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(54) **STABILIZED NON-PROTEIN CLOSTRIDIAL TOXIN COMPOSITIONS**
STABILISIERTE PROTEINFREIE CLOSTRIDIEN TOXINZUSAMMENSETZUNGEN
COMPOSITIONS DE TOXINES CLOSTRIDIALES NON PROTÉIQUES

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Description

FIELD

- 5 **[0001]** The present invention relates to a method of manufacturing a liquid pharmaceutical compositions comprising a botulinum toxin active ingredient and non-protein excipients as defined in the claims.

BACKGROUND

- 10 **[0002]** A pharmaceutical composition is a formulation which contains at least one active ingredient (such as a Clostridial toxin) as well as, for example, one or more excipients, buffers, carriers, stabilizers, preservatives and/or bulking agents, and is suitable for administration to a patient to achieve a desired diagnostic result or therapeutic effect. The pharmaceutical compositions disclosed herein have diagnostic, therapeutic and/or research utility.

- 15 **[0003]** For storage stability and convenience of handling, a pharmaceutical composition can be formulated as a lyophilized (i.e. freeze dried) or vacuum dried powder which can be reconstituted with a suitable fluid, such as saline or water, prior to administration to a patient. Alternately, the pharmaceutical composition can be formulated as an aqueous solution or suspension. A pharmaceutical composition can contain a proteinaceous active ingredient. Unfortunately, a protein active ingredient can be very difficult to stabilize (i.e. maintained in a state where loss of biological activity is minimized), thereby resulting in a loss of protein and/or loss of protein activity during the formulation, reconstitution (if required) and
20 storage of the pharmaceutical composition prior to use. Stability problems can arise due to surface adsorption of a protein active ingredient, physical instability, such as, e.g., denaturation or aggregation, or chemical instability, such as, e.g., crosslinking, deamidation, isomerization, oxidation, formation of acidic or basic species, Maillard reaction, and fragmentation. To prevent such instability, various protein-based excipients, such as albumin and gelatin, have been used to stabilize a protein active ingredient present in a pharmaceutical composition.

- 25 **[0004]** Unfortunately, despite their known stabilizing effects, significant drawbacks exist to the use of protein excipients, such as albumin or gelatin, in a pharmaceutical composition. For example albumin and gelatin are expensive and increasingly difficult to obtain. Furthermore, blood products or animal derived products such as albumin and gelatin, when administered to a patient can subject the patient to a potential risk of receiving blood borne pathogens or infectious agents. Thus, it is known that the possibility exists that the presence of an animal-derived protein excipient in a pharmaceutical composition can result in inadvertent incorporation of infectious elements into the pharmaceutical composition. For example, it has been reported that use of human serum albumin may transmit prions into a pharmaceutical composition. Thus, it is desirable to find suitable non-protein excipients, such as, e.g., stabilizers, cryo-protectants and lyo-protectants, which can be used to stabilize the protein active ingredient present in a pharmaceutical composition.

- 35 **[0005]** The unique characteristics of Clostridial toxins further constrain and hinder the selection of suitable non-protein excipients for a pharmaceutical composition comprising a Clostridial toxin active ingredient. For example, Clostridial toxins are large proteins having an average molecular weight of approximately 150 kDa, and are further complexed with non-toxin associated proteins that increase the size to approximately 300-900-kDa. The size of a Clostridial toxin complex makes it much more fragile and labile than smaller, less complex proteins, thereby compounding the formulation and handling difficulties if Clostridial toxin stability is to be maintained. Hence, the use of non-protein excipients, such as,
40 e.g., stabilizers, cryo-protectants and lyo-protectants must be able to interact with the Clostridial toxin active ingredient in a manner which does not denature, fragment or otherwise inactivate the toxin or cause disassociation of the non-toxin associated proteins present in the toxin complex.

- [0006]** Another problem associated with a Clostridial toxin active ingredient, is the exceptional safety, precision, and accuracy that is necessary for at all steps of the formulation process. Thus, a non-protein excipient should not itself be
45 toxic or difficult to handle so as to not exacerbate the already extremely stringent requirements.

- [0007]** Still another difficulty linked with a Clostridial toxin active ingredient, is the incredible low amounts of Clostridial toxin that is used in a pharmaceutical composition. As with enzymes generally, the biological activities of the Clostridial toxins are dependant, at least in part, upon their three dimensional conformation. Thus, a Clostridial toxin is detoxified by heat, various chemicals, surface stretching, and surface drying. Additionally, it is known that dilution of a Clostridial toxin complex obtained by the known culturing, fermentation and purification methods to the much lower concentration
50 used in a pharmaceutical composition results in rapid inactivation of the toxin. The extremely low amount of a Clostridial toxin active ingredient that is used in a pharmaceutical composition, makes this active ingredient very susceptible to adsorption to, e.g., the surfaces of laboratory glassware, vessels, to the vial in which the pharmaceutical composition is reconstituted and to the inside surface of a syringe used to inject the pharmaceutical composition. Such adsorption
55 of a Clostridial toxin active ingredient to surfaces can lead to a loss of active ingredient and to denaturation of the remaining Clostridial toxin active ingredient, both of which reduce the total activity of the active ingredient present in the pharmaceutical composition. Hence, the use of non-protein excipients, such as, e.g., stabilizers, cryo-protectants and lyo-protectants must be able to act as surface blockers to prevent the adsorption of a Clostridial toxin active ingredient

to a surface.

[0008] Yet another problem connected to a Clostridial toxin active ingredient, is the pH-sensitivity associates with complex formation. For example, the 900-kDa BoNT/A complex is known to be soluble in dilute aqueous solutions at pH 3.5-6.8. However, at a pH above about 7 the non-toxic associated proteins dissociate from the 150-kDa neurotoxin, resulting in a loss of toxicity, particularly as the pH rises above pH 8.0. See Edward J. Schantz et al., pp. 44-45, Preparation and characterization of botulinum toxin type A for human treatment, in Jankovic, J., et al., Therapy with Botulinum Toxin (Marcel Dekker, Inc., 1994). As the non-toxic associated proteins are believed to preserve or help stabilize the secondary and tertiary structures upon which toxicity is depends, the dissociation of these proteins results in a more unstable Clostridial toxin active ingredient. Thus, non-protein excipients useful to formulate a pharmaceutical composition comprising a Clostridial toxin active ingredient must be able to operate within the confines of a pH level necessary to maintain the activity a Clostridial toxin active ingredient.

[0009] WO 2010/090677 discloses animal protein-free, solid-form Clostridial toxin pharmaceutical compositions comprising a Clostridial toxin active ingredient and at least two excipients. US 2016/0250302 discloses methods for treating rhinitis comprising intranasal administration of a topical composition comprising a purified botulinum neurotoxin, a carrier and a viscosity modifier to one or more inner surfaces of the nose. WO 2007/041664 discloses Clostridial toxin pharmaceutical compositions comprising a Clostridial toxin, such as a botulinum toxin, wherein the Clostridial toxin present in the pharmaceutical composition is stabilized by a non-protein excipient such as a polyvinylpyrrolidone, a disaccharide, a trisaccharide, a polysaccharide, an alcohol, a metal, an amino acid, a surfactant and/or a polyethylene glycol. US 2010/0330123 discloses botulinum toxin formulations that are stabilized without the use of any proteinaceous excipients.

[0010] What is needed therefore is a Clostridial toxin pharmaceutical composition wherein a Clostridial toxin active ingredient (such as a botulinum toxin) is stabilized by a non-protein excipient. The present invention relates to a method of manufacturing a liquid botulinum toxin pharmaceutical compositions with non-protein excipients which function to stabilize the botulinum toxin active ingredient present in the liquid pharmaceutical composition.

SUMMARY

[0011] In one aspect of the invention, there is provided a method of manufacturing a liquid pharmaceutical composition as defined in claim 1. In some embodiments, the pharmaceutical composition comprises sodium chloride. The composition of the invention comprises a poloxamer and may additionally comprise a polysorbate. In some embodiments, the composition comprises poloxamer 188 and polysorbate 20. In the invention, the antioxidant is methionine.

[0012] In some embodiments, the liquid pharmaceutical composition further comprises EDTA or an EDTA analog. In some embodiments, the relative weight amount of EDTA ranges from about 0.01% to about 0.05%. In some embodiments, the relative weight amount of poloxamer 188 ranges from about 2% to about 5%.

DESCRIPTION

[0013] The present invention provides a method of manufacturing a stable liquid pharmaceutical composition as defined in the claims.

[0014] Certain embodiments of the present disclosure also provide compositions for use in methods for the treatment of various diseases, disorders, and conditions, including, for example, depression (e.g. major depressive disorder), headache (e.g. migraine, tension headache, and the like), pain, atrial fibrillation, hyperhidrosis, muscle spasticity, cervical dystonia, blepharospasm, overactive bladder (e.g. neurogenic detrusor overactivity, and idiopathic overactive bladder), bladder pain (e.g. interstitial cystitis, bladder pain syndrome), skin conditions (e.g. wrinkles, fine wrinkles, excess sebum production, acne, rosacea), irregularities, and the like using the compositions provided according to the method of the present invention. Embodiments can include various administration techniques, including, for example, injection, such as intramuscular, intracutaneous, subcutaneous, or the like, instillation, intravenous, transdermal, and topical.

Definitions

[0015] As used herein, the words or terms set forth below have the following definitions:

[0016] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0017] "About" or "approximately" as used herein means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, (i.e., the limitations of the measurement system). For example, "about" can mean within 1 or more than 1 standard deviations, per practice in the art. Where particular values are described in the application and claims, unless otherwise stated, the term "about" means within an acceptable error range for the particular value.

[0018] "Administration", or "to administer" means the step of giving (i.e. administering) a pharmaceutical composition

to a subject, or alternatively a subject receiving a pharmaceutical composition. The pharmaceutical compositions disclosed herein can be locally administered by various methods. For example, intramuscular, intradermal, subcutaneous administration, intrathecal administration, intraperitoneal administration, topical (transdermal), instillation, and implantation (for example, of a slow-release device such as polymeric implant or miniosmotic pump) can all be appropriate routes of administration.

[0019] "Alleviating" means a reduction in the occurrence of a pain, of a headache, or of any symptom or cause of a condition or disorder. Thus, alleviating includes some reduction, significant reduction, near total reduction, and total reduction.

[0020] "Animal protein free" means the absence of blood derived, blood pooled and other animal derived products or compounds. "Animal" means a mammal (such as a human), bird, reptile, fish, insect, spider or other animal species. "Animal" excludes microorganisms, such as bacteria. Thus, an animal protein free pharmaceutical composition can include a botulinum neurotoxin. For example, an "animal protein free" pharmaceutical composition means a pharmaceutical composition which is either substantially free or essentially free or entirely free of a serum derived albumin, gelatin and other animal derived proteins, such as immunoglobulins. An example of an animal protein free pharmaceutical composition is a pharmaceutical composition which comprises or which consists of a botulinum toxin (as the active ingredient) and a suitable polysaccharide as a stabilizer or excipient.

[0021] "Antioxidant" refers to any compound which protects an active ingredient from reaction with oxygen. Antioxidants can be broadly divided into three categories: (i) sacrificial antioxidants, which react with oxygen more readily than a particular active ingredient and therefore can scavenge oxygen, e.g., ascorbic acid and sulfites; (ii) chain terminators, which are molecules that form stable radicals due to weak bonds to hydrogen atoms that are attacked in a propagation of radical chains by consumption of oxygen, e.g., methionine, NAC, glutathione, lipoic acid, butylated hydroxytoluene (BHT), and cysteine, (iii) chelating agents, which reduce catalytic activity of transition metals by forming complexes with the metals, e.g., EDTA, EGTA and DTPA and analogs thereof.

[0022] "Biological activity" describes the beneficial or adverse effects of a drug on living matter. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient but can be modified by the other constituents. Biological activity can be assessed as potency or as toxicity by an *in vivo* LD₅₀ or ED₅₀ assay, or through an *in vitro* assay such as, for example, cell-based potency assays (CBPAs) as described in U.S. 20100203559 and U.S. 20100233802. The activities of the compositions of the invention may be measured using any suitable assay and are not limited to activities measured by CBPA. The potency described in the examples also encompasses potency when measured using LD₅₀.

[0023] "Botulinum toxin" means a neurotoxin produced by *Clostridium botulinum*, as well as a botulinum toxin (or the light chain or the heavy chain thereof) made recombinantly by a non-*Clostridial* species. The phrase "botulinum toxin", as used herein, encompasses the botulinum toxin serotypes A, B, C, D, E, F and G, and their subtypes and any other types of subtypes thereof, or any re-engineered proteins, analogs, derivatives, homologs, parts, sub-parts, variants, or versions, in each case, of any of the foregoing. "Botulinum toxin", as used herein, also encompasses a "modified botulinum toxin". Further "botulinum toxin" as used herein also encompasses a botulinum toxin complex, (for example, the 300, 600 and 900kDa complexes), as well as the neurotoxic component of the botulinum toxin (150 kDa) that is unassociated with the complex proteins.

[0024] "Clostridial toxin" refers to any toxin produced by a *Clostridial* toxin strain that can execute the overall cellular mechanism whereby a *Clostridial* toxin intoxicates a cell and encompasses the binding of a *Clostridial* toxin to a low or high affinity *Clostridial* toxin receptor, the internalization of the toxin/receptor complex, the translocation of the *Clostridial* toxin light chain into the cytoplasm and the enzymatic modification of a *Clostridial* toxin substrate. Non-limiting examples of *Clostridial* toxins include a Botulinum toxin like BoNT/A, a BoNT/B, a BoNT/C₁, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G, a Tetanus toxin (TeNT), a Baratii toxin (BaNT), and a Butyricum toxin (BuNT). The BoNT/C₂ cytotoxin and BoNT/C₃ cytotoxin, not being neurotoxins, are excluded from the term "Clostridial toxin." A *Clostridial* toxin disclosed herein includes, without limitation, naturally occurring *Clostridial* toxin variants, such as, e.g., *Clostridial* toxin isoforms and *Clostridial* toxin subtypes; non-naturally occurring *Clostridial* toxin variants, such as, e.g., conservative *Clostridial* toxin variants, non-conservative *Clostridial* toxin variants, *Clostridial* toxin chimeric variants and active *Clostridial* toxin fragments thereof, or any combination thereof. A *Clostridial* toxin disclosed herein also includes a *Clostridial* toxin complex. As used herein, the term "Clostridial toxin complex" refers to a complex comprising a *Clostridial* toxin and non-toxin associated proteins (NAPs), such as, e.g., a Botulinum toxin complex, a Tetanus toxin complex, a Baratii toxin complex, and a Butyricum toxin complex. Non-limiting examples of *Clostridial* toxin complexes include those produced by a *Clostridium botulinum*, such as, e.g., a 900-kDa BoNT/A complex, a 500-kDa BoNT/A complex, a 300-kDa BoNT/A complex, a 500-kDa BoNT/B complex, a 500-kDa BoNT/C₁ complex, a 500-kDa BoNT/D complex, a 300-kDa BoNT/D complex, a 300-kDa BoNT/E complex, and a 300-kDa BoNT/F complex.

[0025] "Clostridial toxin active ingredient" refers to a molecule which contains any part of a *clostridial* toxin that exerts an effect upon or after administration to a subject or patient. As used herein, the term "clostridial toxin active ingredient" encompasses a *Clostridial* toxin complex comprising the approximately 150-kDa *Clostridial* toxin and other proteins

collectively called non-toxin associated proteins (NAPs), the approximately 150-kDa Clostridial toxin alone, or a modified Clostridial toxin, such as, e.g., a re-targeted Clostridial toxins.

[0026] "Deformity" means a cosmetic, physical or functional irregularity, defect, abnormality, imperfection, malformation, depression, or distortion.

[0027] "Effective amount" as applied to the biologically active ingredient means that amount of the ingredient which is generally sufficient to effect a desired change in the subject. For example, where the desired effect is a reduction in an autoimmune disorder symptom, an effective amount of the ingredient is that amount which causes at least a substantial reduction of the autoimmune disorder symptom, and without resulting in significant toxicity.

[0028] "Effective amount" when used in reference to the amount of an excipient or specific combination of excipients added to a Clostridial toxin composition, refers to the amount of each excipient that is necessary to achieve the desired initial recovered potency of a Clostridial toxin active ingredient. In aspects of this embodiment, an effective amount of an excipient or combination of excipients results in an initial recovered potency of, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective concentration of a Clostridial toxin active ingredient reduces a symptom associated with the ailment being treated by, e.g., at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%.

[0029] "Heavy chain" means the heavy chain of a botulinum neurotoxin. It has a molecular weight of about 100kDa and can be referred to as the H chain, or as H.

[0030] H_C means a fragment (about 50kDa) derived from the H chain of a botulinum neurotoxin which is approximately equivalent to the carboxyl end segment of the H chain, or the portion corresponding to that fragment in the intact H chain. It is believed to be immunogenic and to contain the portion of the natural or wild type botulinum neurotoxin involved in high affinity, presynaptic binding to motor neurons.

[0031] H_N means a fragment (about 50kDa) derived from the H chain of a botulinum neurotoxin which is approximately equivalent to the amino end segment of the H chain, or the portion corresponding to that fragment in the intact in the H chain. It is believed to contain the portion of the natural or wild type botulinum neurotoxin involved in the translocation of the L chain across an intracellular endosomal membrane.

[0032] "Light chain" means the light chain of a clostridial neurotoxin. It has a molecular weight of about 50kDa, and can be referred to as the L chain, L, or as the proteolytic domain (amino acid sequence) of a botulinum neurotoxin.

[0033] LH_N or $L-H_N$ means a fragment derived from a clostridial neurotoxin that contains the L chain, or a functional fragment thereof coupled to the H_N domain. It can be obtained from the intact clostridial neurotoxin by proteolysis, so as to remove or to modify the H_C domain.

[0034] "Implant" means a controlled release (e.g., pulsatile or continuous) composition or drug delivery system. The implant can be, for example, injected, inserted or implanted into a human body.

[0035] "Liquid composition", "liquid pharmaceutical composition", or "liquid formulation" refers to a pharmaceutically active preparation of drug or biologically active ingredient which is capable of being stored in a liquid pharmaceutical excipient, such as a buffering agent, for an extended period of time, such that it can be ready-to-use as needed by a clinician. The liquid pharmaceutical composition is manufactured without a lyophilization process.

[0036] "Local administration" means direct administration of a pharmaceutical at or to the vicinity of a site on or within an animal body, at which site a biological effect of the pharmaceutical is desired, such as via, for example, intramuscular or intra- or subdermal injection or topical administration. Local administration excludes systemic routes of administration, such as intravenous or oral administration. Topical administration is a type of local administration in which a pharmaceutical agent is applied to a patient's skin.

[0037] "Lyoprotector" or "lyoprotectant" means a substance that is included in a lyophilized formulation to protect a Clostridial toxin active ingredient during the freeze-drying process. Lyoprotectors include for example polyhydroxy compounds such as sugars (mono-, di-, and polysaccharides), polyalcohols, and their derivatives. Exemplary lyoprotectors which can be used with the lyophilized formulations disclosed herein include sucrose, trehalose, mannitol, sorbitol, glucose, raffinose, maltose, glycerol, lactose, fructose, galactose, and combinations thereof.

[0038] "Lyophilized composition", "lyophilized pharmaceutical composition", "lyophilized formulation", or "solid composition" refers to a formulation containing a Clostridial toxin active ingredient which has been subjected to a lyophilization, freeze-drying or vacuum-drying process; and can be reconstituted with a reconstitution vehicle, such as for example saline or water, prior to administration to a patient. The lyophilized composition can be a freeze-dried composition or a vacuum-dried composition.

[0039] "Modified botulinum toxin" means a botulinum toxin that has had at least one of its amino acids deleted, modified, or replaced, as compared to a native botulinum toxin. Additionally, the modified botulinum toxin can be a recombinantly produced neurotoxin, or a derivative or fragment of a recombinantly made neurotoxin. A modified botulinum toxin retains at least one biological activity of the native botulinum toxin, such as, the ability to bind to a botulinum toxin receptor, or the ability to inhibit neurotransmitter release from a neuron. One example of a modified botulinum toxin is a botulinum toxin that has a light chain from one botulinum toxin serotype (such as serotype A), and a heavy chain from a different

botulinum toxin serotype (such as serotype B). Another example of a modified botulinum toxin is a botulinum toxin coupled to a neurotransmitter, such as substance P.

[0040] "Mutation" means a structural modification of a naturally occurring protein or nucleic acid sequence. For example, in the case of nucleic acid mutations, a mutation can be a deletion, addition or substitution of one or more nucleotides in the DNA sequence. In the case of a protein sequence mutation, the mutation can be a deletion, addition or substitution of one or more amino acids in a protein sequence. For example, a specific amino acid comprising a protein sequence can be substituted for another amino acid, for example, an amino acid selected from a group which includes the amino acids alanine, asparagine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, tyrosine or any other natural or non-naturally occurring amino acid or chemically modified amino acids. Mutations to a protein sequence can be the result of mutations to DNA sequences that when transcribed, and the resulting mRNA translated, produce the mutated protein sequence. Mutations to a protein sequence can also be created by fusing a peptide sequence containing the desired mutation to a desired protein sequence.

[0041] "Patient" means a human or non-human subject receiving medical or veterinary care. Accordingly, the compositions as disclosed herein can be used in treating any animal, such as, for example, mammals, or the like.

[0042] "Peripherally administering" or "peripheral administration" means subdermal, intradermal, transdermal, or subcutaneous administration, but excludes intramuscular administration. "Peripheral" means in a subdermal location, and excludes visceral sites.

[0043] "Pharmaceutical composition" means a composition comprising an active pharmaceutical ingredient, such as, for example, a clostridial toxin active ingredient such as a botulinum toxin, and at least one additional ingredient, such as, for example, a stabilizer or excipient or the like. A pharmaceutical composition is therefore a formulation which is suitable for diagnostic or therapeutic administration to a subject, such as a human patient. The pharmaceutical composition can be, for example, in a lyophilized or vacuum dried condition, a solution formed after reconstitution of the lyophilized or vacuum dried pharmaceutical composition, or as a solution or solid which does not require reconstitution.

[0044] "Pharmacologically acceptable excipient" is synonymous with "pharmacological excipient" or "excipient" and refers to any excipient that has substantially no long term or permanent detrimental effect when administered to mammal and encompasses compounds such as, e.g., stabilizing agent, a bulking agent, a cryo-protectant, a lyo-protectant, an additive, a vehicle, a carrier, a diluent, or an auxiliary. An excipient generally is mixed with an active ingredient, or permitted to dilute or enclose the active ingredient and can be a solid, semi-solid, or liquid agent. It is also envisioned that a pharmaceutical composition comprising a Clostridial toxin active ingredient can include pharmaceutically acceptable excipients that facilitate processing of an active ingredient into pharmaceutically acceptable compositions. Insofar as any pharmacologically acceptable excipient is not incompatible with the Clostridial toxin active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of pharmacologically acceptable excipients can be found in, e.g., *Pharmaceutical Dosage Forms and Drug Delivery Systems* (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed. 1999); *Remington: The Science and Practice of Pharmacy* (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20th ed. 2000); *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and *Handbook of Pharmaceutical Excipients* (Raymond C. Rowe et al., APhA Publications, 4th edition 2003).

[0045] The constituent ingredients of a pharmaceutical composition can be included in a single composition (that is, all the constituent ingredients, except for any required reconstitution fluid, are present at the time of initial compounding of the pharmaceutical composition) or as a two-component system, for example a vacuum-dried composition reconstituted with a reconstitution vehicle which can, for example, contain an ingredient not present in the initial compounding of the pharmaceutical composition. A two-component system can provide several benefits, including that of allowing incorporation of ingredients which are not sufficiently compatible for long-term shelf storage with the first component of the two component system. For example, the reconstitution vehicle may include a preservative which provides sufficient protection against microbial growth for the use period, for example one-week of refrigerated storage, but is not present during the two-year freezer storage period during which time it might degrade the toxin. Other ingredients, which may not be compatible with a botulinum toxin or other ingredients for long periods of time, can be incorporated in this manner; that is, added in a second vehicle (e.g. in the reconstitution vehicle) at the approximate time of use. A pharmaceutical composition can also include preservative agents such as benzyl alcohol, benzoic acid, phenol, parabens and sorbic acid. Pharmaceutical compositions can include, for example, excipients, such as surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; antioxidants; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials and other ingredients known in the art and described, for example in Genaro, ed., 1985, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa..

[0046] "Polysaccharide" means a polymer of more than two saccharide molecule monomers. The monomers can be

identical or different.

[0047] "Stabilizing agent", "stabilization agent" or "stabilizer" means a substance that acts to stabilize a Clostridial toxin active ingredient such that the potency of the pharmaceutical composition is increased relative to an unstabilized composition.

[0048] "Stabilizers" can include excipients, and can include protein and non-protein molecules.

[0049] "Surfactant" refers to a natural or synthetic amphiphilic compound. A surfactant can be non-ionic, zwitterionic, or ionic. Non-limiting examples of surfactants include a poloxamer, a polysorbate, and combinations thereof.

[0050] "Therapeutic formulation" means a formulation can be used to treat and thereby alleviate a disorder or a disease, such as, for example, a disorder or a disease characterized by hyperactivity (*i.e.* spasticity) of a peripheral muscle.

[0051] "TEM" as used herein, is synonymous with "Targeted Exocytosis Modulator" or "retargeted endopeptidase." Generally, a TEM comprises an enzymatic domain from a Clostridial toxin light chain, a translocation domain from a Clostridial toxin heavy chain, and a targeting domain. The targeting domain of a TEM provides an altered cell targeting capability that targets the molecule to a receptor other than the native Clostridial toxin receptor utilized by a naturally-occurring Clostridial toxin. This re-targeted capability is achieved by replacing the naturally-occurring binding domain of a Clostridial toxin with a targeting domain having a binding activity for a non-Clostridial toxin receptor. Although binding to a non-Clostridial toxin receptor, a TEM undergoes all the other steps of the intoxication process including internalization of the TEM/receptor complex into the cytoplasm, formation of the pore in the vesicle membrane and di-chain molecule, translocation of the enzymatic domain into the cytoplasm, and exerting a proteolytic effect on a component of the SNARE complex of the target cell.

[0052] "Tonicity agent" or "isotonic agent" means a low molecular weight excipient which is included in a lyophilized or liquid formulation to provide isotonicity. For example, a disaccharide such as trehalose or sucrose, a polyalcohol such as sorbitol or mannitol, a monosaccharide such as glucose, and a salt such as sodium chloride, can serve as a tonicity agent.

[0053] "Topical administration" excludes systemic administration of the neurotoxin. In other words, and unlike conventional therapeutic transdermal methods, topical administration of botulinum toxin does not result in significant amounts, such as the majority of, the neurotoxin passing into the circulatory system of the patient.

[0054] "Treating" means to alleviate (or to eliminate) at least one symptom of a condition or disorder, such as, for example, wrinkles, spasticity, depression, pain (such as, for example, headache pain), bladder overactivity, or the like, either temporarily or permanently.

[0055] As used herein, the term "unit" or "U" refers to the LD₅₀ dose or the dose determined by a cell based potency assay (CBPA). The LD₅₀ dose is defined as the amount of a Clostridial toxin active ingredient, Clostridial toxin complex or modified Clostridial toxin that killed 50% of the mice injected with the Clostridial toxin, Clostridial toxin complex or modified Clostridial toxin. The CBPA dose is determined as described in US Patent No. 8,618,261.

[0056] "Variant" means a clostridial neurotoxin, such as wild-type botulinum toxin serotype A, B, C, D, E, F or G, that has been modified by the replacement, modification, addition or deletion of at least one amino acid relative to wild-type botulinum toxin, which is recognized by a target cell, internalized by the target cell, and catalytically cleaves a SNARE (SNAP (Soluble NSF Attachment Protein) Receptor) protein in the target cell.

[0057] An example of a variant neurotoxin component can comprise a variant light chain of a botulinum toxin having one or more amino acids substituted, modified, deleted and/or added. This variant light chain may have the same or better ability to prevent exocytosis, for example, the release of neurotransmitter vesicles. Additionally, the biological effect of a variant may be decreased compared to the parent chemical entity. For example, a variant light chain of a botulinum toxin type A having an amino acid sequence removed may have a shorter biological persistence than that of the parent (or native) botulinum toxin type A light chain.

[0058] Sugar is defined in the next section. As used herein, the term "polyalcohol" is synonymous with "sugar alcohol," "polyhydric alcohol," and "polyol" and refers to a sugar derivative having an alcohol group (CH₂OH) instead of the aldehyde group (CHO), such as, e.g., mannitol from mannose, xylitol from xylose, and lactitol from lactulose. Non-limiting examples of polyols include, glycol, glycerol, arabitol, erythritol, xylitol, maltitol, sorbitol (gluctiol), mannitol, inositol, lactitol, galactitol (iditol), isomalt. Other non-limiting examples of sugar excipients can be found in, e.g., Ansel, supra, (1999); Gennaro, supra, (2000); Hardman, supra, (2001); and Rowe, supra, (2003).

Pharmaceutical compositions

[0059] Certain embodiments of the present invention include a method of manufacturing a liquid pharmaceutical composition as defined in claim 5.

[0060] As used herein, the term "therapeutically effective concentration" is synonymous with "therapeutically effective amount," "effective amount," "effective dose," and "therapeutically effective dose" and refers to the minimum dose of a botulinum toxin necessary to achieve the desired therapeutic effect and includes a dose sufficient to reduce a symptom associated with ailment being treated. In aspects of this embodiment, a therapeutically effective concentration of a

botulinum toxin reduces a symptom associated with the aliment being treated by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective concentration of a botulinum toxin reduces a symptom associated with the aliment being treated by, e.g., at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%.

[0061] It is envisioned that any amount of botulinum toxin can be added in formulating the pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the toxin active ingredient is recoverable. In aspects of this embodiment, the amount of botulinum toxin added to the formulation is at least 0.1 U/ml, at least 1.0 U/ml, at least 10 U/ml, at least 50 U/ml, at least 100 U/ml, at least 200 U/ml, or at least 1000 U/ml. In other aspects of this embodiment, the amount of botulinum toxin added to the formulation is at most 0.1 U/ml, at most 1.0 U/ml, at most 10 U/ml, at most 50 U/ml, at most 100 U/ml, at most 200 U/ml, or at most 1000 U/ml. In yet other aspects of this embodiment, the amount of botulinum toxin added to the formulation is from about 0.1 U/ml to about 1000 U/ml, or about 1.0 U/ml to about 1000 U/ml. In still other aspects of this embodiment, the amount of botulinum toxin added to the formulation is from about 0.001 U/ml to about 100 U/ml, about 0.01 U/ml to about 100 U/ml, about 0.1 U/ml to about 100 U/ml, or about 1.0 U/ml to about 100 U/ml.

[0062] In other aspects of this embodiment, the amount of botulinum toxin added to the formulation is at least 1.0 pg, at least 10 pg, at least 100 pg, at least 1.0 ng, at least 10 ng, at least 100 ng, at least 1.0 μ g, at least 10 μ g, at least 100 μ g, or at least 1.0 mg. In still other aspects of this embodiment, the amount of botulinum toxin added to the formulation is at most 1.0 pg, at most 10 pg, at most 100 pg, at most 1.0 ng, at most 10 ng, at most 100 ng, at most 1.0 μ g, at most 10 μ g, or at most 1.0 mg. In still other aspects of this embodiment, the amount of botulinum toxin added to the formulation is about 1.0 pg to about 10 μ g, about 10 pg to about 10 μ g, about 100 pg to about 10 μ g, about 1.0 ng to about 10 μ g, about 10 ng to about 10 μ g, or about 100 ng to about 10 μ g. In still other aspects of this embodiment, the amount of botulinum toxin added to the formulation is about 1.0 pg to about 1.0 μ g, about 10 pg to about 1.0 μ g, about 100 pg to about 1.0 μ g, about 1.0 ng to about 1.0 μ g, about 10 ng to about 1.0 μ g, or about 100 ng to about 1.0 μ g. In further aspects of this embodiment, the amount of botulinum toxin added to the formulation is about 1.0 pg to about 5.0 μ g, about 10 pg to about 5.0 μ g, about 100 pg to about 5.0 μ g, about 1.0 ng to about 5.0 μ g, about 10 ng to about 5.0 μ g, or about 100 ng to about 5.0 μ g. In further aspects of this embodiment, the amount of botulinum toxin added to the formulation is about 1.0 pg to about 10 μ g, about 10 pg to about 10 μ g, about 100 pg to about 10 μ g, about 1.0 ng to about 10 μ g, about 10 ng to about 10 μ g, or about 100 ng to about 10 μ g.

[0063] In aspects of this embodiment, the toxin pharmaceutical composition comprises a BoNT/A, a BoNT/B, a BoNT/C₁, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G, or a BoNT mosaic, such as BoNT/DC, BoNT/CD and BoNT/FA. In another embodiment, a pharmaceutical composition comprises a toxin variant as the toxin active ingredient. In aspects of this embodiment, a toxin pharmaceutical composition comprises naturally-occurring toxin variant or a non-naturally-occurring toxin variant. In other aspects of this embodiment, a toxin pharmaceutical composition comprises a BoNT/A variant, a BoNT/B variant, a BoNT/C₁ variant, a BoNT/D variant, a BoNT/E variant, a BoNT/F variant, or a BoNT/G variant, where the variant is either a naturally-occurring variant or a non-naturally-occurring variant.

[0064] As used herein, the term "botulinum toxin complex" refers to a complex comprising a botulinum toxin and associated NAPs as a Botulinum toxin complex. Non-limiting examples of toxin complexes include those produced by a Clostridium botulinum, such as, e.g., a 900-kDa BoNT/A complex, a 500-kDa BoNT/A complex, a 300-kDa BoNT/A complex, a 500-kDa BoNT/B complex, a 500-kDa BoNT/C₁ complex, a 500-kDa BoNT/D complex, a 300-kDa BoNT/D complex, a 300-kDa BoNT/E complex, and a 300-kDa BoNT/F complex. Toxin complexes can be purified using the methods described in Schantz, supra, (1992); Hui Xiang et al., Animal Product Free System and Process for Purifying a Botulinum Toxin, U.S. Patent 7,354,740. Toxin complexes can be obtained from, e.g., List Biological Laboratories, Inc. (Campbell, California), the Centre for Applied Microbiology and Research (Porton Down, U.K), Wako (Osaka, Japan), and Sigma Chemicals (St Louis, Missouri).

[0065] As used herein, the term "non-protein excipient" refers to any excipient that is not a polypeptide comprising at least fifteen amino acids. It is envisioned that any non-protein excipient is useful in formulating the toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the botulinum toxin is recovered using this non-protein excipient.

[0066] As used herein, the term "sugar" refers to a compound comprising one to 10 monosaccharide units, e.g., a monosaccharide, a disaccharide, a trisaccharide, and an oligosaccharide comprising four to ten monosaccharide units. Trehalose is present in the liquid composition manufactured according to the invention, and it is envisioned that any further sugar is useful in formulating the pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the botulinum toxin is recovered using this sugar. In some embodiments, the sugar can function as a tonicity agent. Monosaccharides are polyhydroxy aldehydes or polyhydroxy ketones with three or more carbon atoms, including aldoses, dialdoses, aldoketoses, ketoses and diketoses, as well as cyclic forms, deoxy sugars and amino sugars, and their derivatives, provided that the parent monosaccharide has a (potential) carbonyl group. Monosaccharides include trioses, like glyceraldehyde and dihydroxyacetone; tetroses, like erythrose, erythrulose

and threose; pentoses, like arabinose, lyxose, ribose, ribulose, xylose, xylulose; hexoses, like allose, altrose, fructose, fucose, galactose, glucose, gulose, idose, mannose, psicose, rhamnose, sorbose, tagatose, talose and trehalose; heptoses, like sedoheptulose and mannoheptulose; octoses, like octulose and 2-keto-3-deoxy-manno-octonate; nonoses like sialose; and decose. Oligosaccharides are compounds in which at least two monosaccharide units are joined by glycosidic linkages. According to the number of units, they are called disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexoaccharides, heptoaccharides, octoaccharides, nonoaccharides, decoaccharides, etc. An oligosaccharide can be unbranched, branched or cyclic. Common disaccharides include, without limitation, sucrose, lactose, maltose, trehalose, cellobiose, gentiobiose, kojibiose, laminaribiose, mannobiose, melibiose, nigerose, rutinose, and xylobiose. Common trisaccharides include, without limitation, raffinose, acarbose, maltotriose, and melezitose. Other non-limiting examples of specific uses of sugar excipients can be found in, e.g., Ansel, supra, (1999); Gennaro, supra, (2000); Hardman, supra, (2001); and Rowe, supra, (2003).

[0067] The pharmaceutical composition manufactured according to the invention comprises trehalose. In aspects of this embodiment, the toxin pharmaceutical composition further comprises a monosaccharide. In other aspects of this embodiment, the pharmaceutical composition further comprises a further disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexoaccharide, a heptoaccharide, an octoaccharide, a nonoaccharide, or a decoaccharide. In yet other aspects of this embodiment, the toxin pharmaceutical composition comprises an oligosaccharide comprising two to ten monosaccharide units. Trehalose is used in the invention, and sucrose is a particularly preferred further sugars for use in the invention.

[0068] Trehalose is useful in the method for manufacturing the liquid pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the botulinum toxin is recovered using this sugar amount. In aspects of this embodiment, the amount of sugar, which is preferably trehalose, added to the formulation is about 1.0% (w/w), about 1.5% (w/w), about 2.0% (w/w), about 2.5% (w/w), about 3.0% (w/w), about 3.5% (w/w), about 4.0% (w/w), about 4.5% (w/w), about 5.0% (w/w), about 5.5% (w/w), about 6.0% (w/w), about 6.5% (w/w), about 7.0% (w/w), about 7.5% (w/w), about 8.0% (w/w), about 8.5% (w/w), about 9.0% (w/w), about 9.5% (w/w), about 10% (w/w). In other aspects of this embodiment, the amount of sugar, which is trehalose, added to the formulation is at least 1.5% (w/w), at least 2.0% (w/w), at least 2.5% (w/w), at least 3.0% (w/w), at least 3.5% (w/w), at least 4.0% (w/w), at least 4.5% (w/w), at least 5.0% (w/w), at least 5.5% (w/w), at least 6.0% (w/w), at least 6.5% (w/w), at least 7.0% (w/w), at least 7.5% (w/w), at least 8.0% (w/w), at least 8.5% (w/w), at least 9.0% (w/w), at least 9.5% (w/w) however not more than 10% (w/w). In yet other aspects of this embodiment, the amount of sugar, which is trehalose, added to the formulation is at most 1.5% (w/w), at most 2.0% (w/w), at most 2.5% (w/w), at most 3.0% (w/w), at most 3.5% (w/w), at most 4.0% (w/w), at most 4.5% (w/w), at most 5.0% (w/w), at most 5.5% (w/w), at most 6.0% (w/w), at most 6.5% (w/w), at most 7.0% (w/w), at most 7.5% (w/w), at most 8.0% (w/w), at most 8.5% (w/w), at most 9.0% (w/w), at most 9.5% (w/w), however not less than 1% (w/w).

[0069] Common disaccharides include, without limitation, sucrose, lactose, maltose, trehalose, cellobiose, gentiobiose, kojibiose, laminaribiose, mannobiose, melibiose, nigerose, rutinose, and xylobiose.

[0070] As used hereon, the term "surfactant" refers to a natural or synthetic amphiphilic compound. A surfactant can be non-ionic, zwitterionic, or ionic. Poloxamer 188 (PLURONIC® F68) is used in the present invention. Other examples of surfactants include polysorbates like polysorbate 20 (TWEEN® 20), polysorbate 40 (TWEEN® 40), polysorbate 60 (TWEEN® 60), polysorbate 61 (TWEEN® 61), polysorbate 65 (TWEEN® 65), polysorbate 80 (TWEEN® 80), and polysorbate 81 (TWEEN® 81); poloxamers (polyethylene-polypropylene copolymers), like Poloxamer 124 (PLURONIC® L44), Poloxamer 181 (PLURONIC® L61), Poloxamer 182 (PLURONIC® L62), Poloxamer 184 (PLURONIC® L64), Poloxamer 237 (PLURONIC® F87), Poloxamer 338 (PLURONIC® L108), Poloxamer 407 (PLURONIC® F127), polyoxyethyleneglycol dodecyl ethers, like BRIJ® 30, and BRIJ® 35; 2-dodecoxyethanol (LUBROL®-PX); polyoxyethylene octyl phenyl ether (TRITON® X-100); sodium dodecyl sulfate (SDS); solutol HS15; 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS); 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO); sucrose monolaurate; and sodium cholate. Other non-limiting examples of surfactant excipients can be found in, e.g., Ansel, supra, (1999); Gennaro, supra, (2000); Hardman, supra, (2001); and Rowe, supra, (2003).

[0071] Thus in the invention, a pharmaceutical composition comprises a surfactant, which is Poloxamer 188.

[0072] Poloxamer 188 is useful in formulating the toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the botulinum toxin is recovered using this surfactant amount. In aspects of this embodiment, the amount of Poloxamer 188 added to the formulation is about 0.5% (w/w), about 1.0% (w/w), about 1.5% (w/w), about 2.0% (w/w), about 2.5% (w/w), about 3.0% (w/w), about 3.5% (w/w), about 4.0% (w/w), about 4.5% (w/w), about 5.0% (w/w). In other aspects of this embodiment, the amount of Poloxamer 188 added to the formulation is at least 0.5% (w/w), at least 1.0% (w/w), at least 1.5% (w/w), at least 2.0% (w/w), at least 2.5% (w/w), at least 3.0% (w/w), at least 3.5% (w/w), at least 4.0% (w/w), at least 4.5% (w/w), however no more than 5% (w/w). In yet other aspects of this embodiment, the amount of Poloxamer 188 added to the formulation is at most 1.0% (w/w), at most 1.5% (w/w), at most 2.0% (w/w), at most 2.5% (w/w), at most 3.0% (w/w), at most 3.5% (w/w), at most 4.0% (w/w), at most 4.5% (w/w), at most 5.0% (w/w), however no less than 0.5 % (w/w).

[0073] The present pharmaceutical compositions manufactured according to the invention comprise the antioxidant methionine. Other examples of antioxidant include, without limitation, cysteine, N-acetyl-cysteine (NAC), sodium metabisulfite, sodium thiosulfate, butylated hydroxyanisole, butylated hydroxytoluene, vitamin E and analogs including Trolox C; chelators such as EDTA (ethylene diamine tetraacetic acid sodium salt), EGTA (ethylene glycol-bis(2-aminoethyl-
 5 ether)-N,N,N',N'-tetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), analogs or derivatives thereof, and combinations thereof. In formulations of the invention, methionine is the sole antioxidant used. In the invention, the amount of methionine added to the formulation ranges from 0.1% (w/w) to 0.3% (w/w).

[0074] It is further envisioned that a pharmaceutical composition disclosed in the present specification can optionally include, without limitation, other pharmaceutically acceptable components (or pharmaceutical components), including, without limitation, preservatives, tonicity adjusters, salts, antioxidants, osmolality adjusting agents, emulsifying agents, sweetening or flavoring agents, and the like. The buffer is histidine in the invention. It is understood that acids or bases can be used to adjust the pH of a pharmaceutical composition as needed. The buffered pH level used in formulating the pharmaceutical composition, with the proviso that a therapeutically effective amount of the toxin active ingredient, which is a botulinum toxin, is recovered using this effective pH level. In an aspect of this embodiment, an effective pH level is
 10 at least about pH 5.5, at least about pH 6.0, at least about pH 6.5, but is no more than 7. In another aspect of this embodiment, an effective pH level is at most about pH 5.0, at most about pH 5.5, at most about pH 6.0, at most about pH 6.5, or at most about pH 7.0, but is no less than 5. In yet another aspect of this embodiment, an effective pH level is about pH 5.0 to about pH 6.0, an effective pH level is about pH 5.5 to about pH 7.0, an effective pH level is about pH 5.5 to about pH 5.0, an effective pH level is about pH 5.5 to about pH 6.5. An effective pH of 5.5 to 6.0 is particularly
 20 preferred.

[0075] In certain embodiments the composition manufactured according to the invention will have a pH of, for example, 7.0, or 6.9, or 6.8, or 6.7, or 6.6, or 6.5, or 6.4, or 6.3, or 6.2, or 6.1, or 6.0, or 5.9, or 5.8, or 5.7, or 5.6, or 5.5, or 5.4, or 5.3, or 5.2, or 5.1. A pH of 5.5 to 6.5 is preferred and a pH of 5.5 to 6.0 is particularly preferred.

[0076] It is envisioned that any concentration of a buffer can be useful in formulating the pharmaceutical composition, with the proviso that a therapeutically effective amount of the botulinum toxin is recovered using this effective concentration of buffer, which is histidine. In aspects of this embodiment, an effective concentration of buffer, which is histidine, is at least 0.1 mM, at least 0.2 mM, at least 0.3 mM, at least 0.4 mM, at least 0.5 mM, at least 0.6 mM, at least 0.7 mM, at least 0.8 mM, or at least 0.9 mM. In other aspects of this embodiment, an effective concentration of buffer, which is histidine, is at least 1.0 mM, at least 2.0 mM, at least 3.0 mM, at least 4.0 mM, at least 5.0 mM, at least 6.0 mM, at least 7.0 mM, at least 8.0 mM, or at least 9.0 mM. In yet other aspects of this embodiment, an effective concentration of buffer, which is histidine, is at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, or at least 90 mM. In still other aspects of this embodiment, an effective concentration of buffer, which is histidine, is at least 100 mM, at least 200 mM, at least 300 mM, at least 400 mM, at least 500 mM, at least 600 mM, at least 700 mM, at least 800 mM, or at least 900 mM. In further aspects of this embodiment, an effective
 35 concentration of buffer, which is histidine, is at most 0.1 mM, at most 0.2 mM, at most 0.3 mM, at most 0.4 mM, at most 0.5 mM, at most 0.6 mM, at most 0.7 mM, at most 0.8 mM, or at most 0.9 mM. In still other aspects of this embodiment, an effective concentration of buffer, which is histidine, is at most 1.0 mM, at most 2.0 mM, at most 3.0 mM, at most 4.0 mM, at most 5.0 mM, at most 6.0 mM, at most 7.0 mM, at most 8.0 mM, or at most 9.0 mM. In yet other aspects of this embodiment, an effective concentration of buffer, which is histidine, is at most 10 mM, at most 20 mM, at most 30 mM, at most 40 mM, at most 50 mM, at most 60 mM, at most 70 mM, at most 80 mM, or at most 90 mM. In still other aspects
 40 of this embodiment, an effective concentration of buffer, which is histidine, is at most 100 mM, at most 200 mM, at most 300 mM, at most 400 mM, at most 500 mM, at most 600 mM, at most 700 mM, at most 800 mM, or at most 900 mM. In still further aspects of this embodiment, an effective concentration of buffer, which is histidine, is about 0.1 mM to about 900 mM, 0.1 mM to about 500 mM, 0.1 mM to about 100 mM, 0.1 mM to about 90 mM, 0.1 mM to about 50 mM, 1.0 mM to about 900 mM, 1.0 mM to about 500 mM, 1.0 mM to about 100 mM, 1.0 mM to about 90 mM, or 1.0 mM to about 50 mM. The concentration of the buffer, which is histidine, is preferably 20 mM.

[0077] Embodiments of the invention can be practiced with a composition that comprises a plurality of botulinum toxin serotypes, such as botulinum toxin serotypes selected from the group consisting of botulinum toxin serotypes A, B, C₁, D, E, F, G and mosaic. In certain embodiments, purified botulinum toxins, can be used. In other embodiments, modified
 50 botulinum toxins may be used.

[0078] In the invention, the pharmaceutical composition can be formulated as an aqueous solution or suspension.

[0079] The combination of poloxamer 188 with methionine in the present invention is used because of the synergistic effect on the stability of the compositions caused by the combination of this particular surfactant with methionine.

[0080] The composition manufactured by the method of the invention comprises trehalose.

[0081] In addition, the composition manufactured by the method of the invention may optionally also include NaCl. NaCl may particularly preferably be included in compositions comprising botulinum toxin, trehalose, poloxamer 188, and methionine, and is particularly preferably included in liquid compositions comprising botulinum toxin, trehalose, poloxamer 188, and methionine.
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[0082] In addition to or instead of NaCl, the composition may optionally include EDTA, EGTA, DTPA, or analogs thereof. EDTA may be included in compositions comprising botulinum toxin, trehalose, poloxamer 188 and methionine, and is preferably included in liquid compositions comprising botulinum toxin, trehalose, poloxamer 188 and methionine.

[0083] The relative weight amounts (% w/w) of trehalose, poloxamer 188, methionine, and NaCl where present, and EDTA where present are within the following ranges:

Trehalose or sucrose 1 to 10

poloxamer 188 0.5 to 5

methionine 0.1 to 0.3

NaCl 0.1 to 10

EDTA 0.01 to 0.1. Such compositions are liquid formulations.

[0084] In addition, these compositions comprise a buffer. In compositions of the invention, the buffer is histidine, and the concentration of histidine is preferably 20 mM. In these compositions, the pH is in the range 5-7, the pH is more preferably in the range 5.5-6.5, and the pH is most preferably in the range 5.5-6.

[0085] More preferably, the relative weight amounts (% w/w) of trehalose, poloxamer 188, and methionine are:

a)	trehalose	7 to 9
	poloxamer 188	3.5 to 4.5
	methionine	0.15 to 0.25;

and are preferably		
	trehalose	7 to 9
	poloxamer 188	3.5 to 4.5
	methionine	0.15 to 0.25;

[0086] In the invention, the composition labelled as a) is a liquid composition manufactured according to the method of the invention. In this case, the composition comprises a buffer, wherein the buffer is histidine. The concentration of histidine is preferably 20 mM, and the pH is in the range 5-7, more preferably in the range 5.5-6.5, and the pH is yet more preferably in the range 5.5-6, and is most preferably 6.

[0087] In the invention, the composition manufactured according to the method of the invention is a liquid composition consisting of one or more botulinum toxins, trehalose, poloxamer 188, methionine, and a buffer, wherein these components are present in the amounts defined in the claims.

[0088] Also more preferably, the relative weight amounts (% w/w) of trehalose, poloxamer 188 and methionine are:

b)	trehalose	7 to 9
	poloxamer 188	0.5 to 0.7
	methionine	0.15 to 0.25;

and are preferably		
	trehalose	8
	poloxamer 188	0.6
	methionine	0.2

[0089] In the invention, the composition labelled as b) above is a liquid composition. In this case, the composition comprises a buffer, wherein the buffer is histidine. The concentration of histidine is preferably 20 mM, and the pH is in the range 5-7, the pH is more preferably in the range 5.5-6.5, and the pH is yet more preferably in the range 5.5-6, and is most preferably 5.5.

[0090] In one embodiment of the invention, the composition is a liquid composition consisting of one or more botulinum toxins, trehalose, poloxamer 188, methionine, and a buffer, wherein these components are present in the amounts defined in the claims.

[0091] Also more preferably, the relative weight amounts (% w/w) of trehalose, poloxamer 188, methionine, and NaCl are:

c)	trehalose	1 to 3
	poloxamer 188	3.5 to 4.5
	methionine	0.15 to 0.25
	NaCl	0.4 to 0.8;

and are preferably

trehalose	2
poloxamer 188	4
methionine	0.2
NaCl	0.6

[0092] In the invention, the composition labelled as c) above is a liquid composition. In this case, the composition comprises a buffer, wherein the buffer is histidine. The concentration of histidine is preferably 20 mM, and the pH is in the range 5-7, and the pH is more preferably in the range 5.5-6.5, the pH is yet more preferably in the range 5.5-6, and is most preferably 5.5.

[0093] In one embodiment of the invention, the composition is a liquid composition consisting of one or more botulinum toxins, trehalose, poloxamer 188, methionine, NaCl and a buffer, wherein these components are present in the amounts defined in the claims.

[0094] Also more preferably, the relative weight amounts (% w/w) of trehalose, poloxamer 188, methionine, and EDTA are:

d)	trehalose	7 to 9
	poloxamer 188	3.5 to 4.5
	methionine	0.15 to 0.25
	EDTA	0.01 to 0.05;

and are preferably

trehalose	8
poloxamer 188	4
methionine	0.2
EDTA	0.03.

[0095] In the invention, the composition labelled as d) above is a liquid composition. In this case, the composition comprises a buffer, wherein the buffer is histidine. The concentration of histidine is preferably 20 mM, and the pH is in the range 5-7, the pH is more preferably in the range 5.5-6.5, and the pH is yet more preferably in the range 5.5-6, and is most preferably 6.

[0096] In one embodiment of the invention, the composition is a liquid composition consisting of one or more botulinum toxins, trehalose, poloxamer 188, methionine, EDTA and a buffer, wherein these components are present in the amounts defined in the claims.

[0097] The compositions of the invention are liquid compositions, in which case the composition comprises a buffer. This buffer is histidine, and the concentration of histidine is preferably 20 mM. The pH is in the range 5-7, the pH is more preferably in the range 5.5-6.5, and is most preferably in the range 5.5-6.

[0098] It is particularly preferred that the composition of the invention comprises no animal-derived protein.

[0099] Any of the foregoing compositions comprising a botulinum toxin, poloxamer 188, and methionine may also further comprise ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog. The relative weight amount (% w/w) of EDTA in these compositions may be in the range from about 0.01 to 0.10.

[0100] In the present invention, the term "Antioxidant" refers to any compound which protects an active ingredient from reaction with oxygen. Antioxidants can be broadly divided into three categories:

(i) sacrificial antioxidants, which react with oxygen more readily than a particular active ingredient and therefore can scavenge oxygen. In the invention, this type of antioxidant is preferably ascorbic acid;

(ii) chain terminators, which react with free radicals and peroxides to prevent propagation of radical chains. In the invention, methionine is used as a chain terminator; and

(iii) chelating agents, which reduce catalytic activity of transition metals by forming complexes with the metals. In the invention, EDTA, EGTA, and DTPA are preferred chelating agents.

Compositions for use in methods of treatment

[0101] In embodiments of the present disclosure, compositions for use in methods of treating diseases, disorders, conditions, and the like are provided, comprising the step of administering a pharmaceutical formulation of the invention to a subject in need thereof in an amount sufficient to produce improved patient function. In certain embodiments, the diseases are of a neuromuscular nature, such as, for example, those diseases that affect muscles and nerve control thereof, such as, for example, overactive bladder, and the like. In preferred compositions for use in a method, the compositions of the invention are used in the treatment of cardiac arrhythmia. Certain embodiments relate to the treatment of pain, such as, for example, treatment of headache pain, or back pain, or muscle pain, or the like. In certain embodiments, compositions for use in methods of the invention encompass the treatment of psychological disorders, including, for example, depression, anxiety, and the like. In preferred compositions for use in a method, the compositions of the invention are used in the treatment of depression.

[0102] Where the composition is used in the treatment of depression, it is preferably used to treat adults with moderate to severe major depressive disorder (MDD) either single episode or recurrent, wherein this diagnosis is based upon the Diagnostic and Statistical Manual of Mental Disorders Text Edition, published in 2000 (DSM-IV-TR). Any of the compositions of the invention can be used. The following composition in Table A does not form part of the invention. Table A:

Table A

Raw Material (Ingredient)	Concentration	Quantity per vial	Function
Botulinum toxin type A	1200 U/mL	200U	Drug Substance
Trehalose	80 mg/mL	16 mg	Lyo Protectant
P188	40 mg/mL	8 mg	Surfactant
L-methionine	2 mg/mL	0.4 mg	Antioxidant
L-Histidine	1.430 mg/mL	0.286 mg	Buffer
L-Histidine HCl, Monohydrate	2.263 mg/mL	0.453 mg	Buffer
Water	QS Adjust	QS Adjust	Solvent, removed by freeze-drying

[0103] In one embodiment according to the present disclosure, the composition is reconstituted with a reconstitution vehicle comprising NaCl prior to administration to a patient. In one embodiment according to the present disclosure, NaCl may be present in an amount of 0.9% (w/w) in the reconstitution vehicle.

[0104] The composition is preferably administered in a single treatment session. The dosage is preferably a total dose of 30 Units or 50 Units. These dosages are preferably administered in divided injection into 6 (for the 30 units dosage) or 8 (for the 50 Units dosage) injection sites into the glabellar region of the forehead, preferably into the procerus and corrugator muscles. This treatment may be repeated in 3 to 6 months intervals. One advantage of the use of this method is that the patients treated with this composition show an improvement in the clinic Montgomery-Asberg Depression Rating scale. In addition, these patients also have improved clinic CGI-S scores (Clinical Global Impression of Change scores), and clinic HAM-D17 total scores (Hamilton Rating Scale for Depression).

[0105] Compositions and compositions for use in methods of the present disclosure can be useful for the treatment, reduction of symptoms, and/or prevention of, for example, achalasia, anal fissure, anismus, blepharospasm, cerebral palsy, cervical dystonia, cervicogenic headache, hemifacial spasm, dyshidrotic eczema, dysphagia, dysphonia, esophageal dysmotility, esophageal muscular ring, esotropia (infantile), eyelift, facial myokemia, gait disturbances (idiopathic toe-walking), generalized dystonia, hemifacial spasm, hyperfunctional facial lines (glabellar, forehead, crows' feet, downturned angles of the mouth), hyperhidrosis, incontinence (idiopathic or neurogenic), medication overuse headache, migraine headache, myoclonus, muscle mass or activity reduction, involving, for example, the masseter or the like, myofascial pain syndrome, obstructive urinary symptoms, pancreas divisum pancreatitis, Parkinson's disease, puborectalis syndrome, reduction of surgical scar tension, salivary hypersecretion, sialoceles, sixth nerve palsy, spasticity, speech/voice disorders, strabismus, surgery adjunct (ophthalmic), tardive dyskinesia, temporomandibular joint disorders, tension headache, thoracic outlet syndrome, torsion dystonia, torticollis, Tourette's syndrome, tremor, whiplash-associated neck pain, pain, itching, inflammation, allergy, cancer and benign tumors, fever, obesity, infectious diseases, viral and bacterial, hypertension, cardiac arrhythmias, vasospasm, atherosclerosis, endothelial hyperplasia, venous thrombosis, varicose veins, aphthous stomatitis, hypersalivation, temporomandibular joint syndrome, hyperhidrosis, bromhidrosis, acne, rosacea, hyperpigmentation, hypertrophic scars, keloids, calluses and corns, skin wrinkling, excessive sebum production, psoriasis, dermatitis, allergic rhinitis, nasal congestion, post nasal drip, sneezing, ear wax, serous and suppurative otitis media, tonsil and adenoid hypertrophy, tinnitus, dizziness, vertigo, hoarseness, cough, sleep apnea,

snoring, glaucoma, conjunctivitis, uveitis, strabismus, Grave's disease, excessive hair growth, hair loss, asthma, bronchitis, emphysema, mucus production, pleuritis, coagulation disorders, myeloproliferative disorders, disorders involving eosinophils, neutrophils, macrophages and lymphocytes, immune tolerance and transplantation, autoimmune disorders, dysphagia, acid reflux, hiatal hernia, gastritis and hyperacidity, diarrhea and constipation, hemorrhoids, urinary incontinence, prostatic hypertrophy, erectile dysfunction, priapism and Peyronie's disease, epididymitis, contraception, menstrual cramps, preventing premature delivery, endometriosis and fibroids, arthritis, osteoarthritis, rheumatoid, bursitis, tendonitis, tenosynovitis, fibromyalgia, seizure disorders, spasticity, headache, and neuralgias. In preferred compositions for use in a method, the compositions of the invention are used in the treatment of cardiac arrhythmia.

[0106] Where the composition is used in the treatment of cardiac arrhythmia, the patient is typically undergoing cardiac surgery. The formulation used in the treatment may be any of the compositions of the invention. The following composition in Table A does not form part of the invention. Table A:

Table A

Raw Material (Ingredient)	Concentration	Quantity per vial	Function
Botulinum toxin type A	1200 U/mL	200U	Drug Substance
Trehalose	80 mg/mL	16 mg	Lyo Protectant
P188	40 mg/mL	8 mg	Surfactant
L-methionine	2 mg/mL	0.4 mg	Antioxidant
L-Histidine	1,430 mg/mL	0.286 mg	Buffer
L-Histidine HCl, Monohydrate	2.263 mg/mL	0.453 mg	Buffer
Water	QS Adjust	QS Adjust	Solvent, removed by freeze-drying

[0107] In one embodiment according to the present disclosure, the composition is reconstituted with a reconstitution vehicle comprising NaCl prior to administration to a patient. In one embodiment according to the present disclosure, NaCl may be present in an amount of 0.9% (w/w) in the reconstitution vehicle.

[0108] The mode of administration of the composition is by one time injection into the major epicardial fat pads of the heart. The dosage used is 25 U per epicardial fat pad (125 U total dose) or 50 U per epicardial fat pad (250 U total dose). One advantage of the use of this method is that the incidence of atrial fibrillation (AF) as measured by ECG through week 4 can be reduced. Other advantages include: a reduction in the length of hospital stay; reduction in the length of stay in ICU; reduced rehospitalization rate of patients; reduced anticoagulant medication use; and reduced need for interventional procedures for Post Operative Atrial fibrillation (POAF), such as ablation, pacemaker implantation, electrical or pharmacologic cardioversion.

[0109] In certain embodiments according to the present disclosure, patients are limited to a maximum of 360U of botulinum toxin administered over any 90-day period.

Treatment of nerve / muscle conditions

[0110] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0111] In an embodiment, the neuromuscular disease is hyperhidrosis. A subject suffering from hyperhidrosis, for example, receives about 59U per axilla, or about 58U per axilla, or about 57U per axilla, or about 56U per axilla, or about 55U per axilla, or about 54U per axilla, or about 53U per axilla, or about 52U per axilla, or about 51U per axilla, or about 50U per axilla, or about 49U per axilla, or about 48U per axilla, or about 47U per axilla, or about 46U per axilla, or about 45U per axilla, or about 44U per axilla, or about 43U per axilla, or about 42U per axilla, or about 41U per axilla, or about 40U per axilla, or about 39U per axilla, or about 38U per axilla, or about 37U per axilla, or about 36U per axilla, or less, per treatment of a pharmaceutical formulation of the present invention. In an embodiment, 50U total are injected intradermally into 10-15 sites spaced approximately 1-2cm apart.

[0112] In an embodiment, the neuromuscular disease is hemifacial spasm. A subject suffering from hemifacial spasm, for example receives between about 1.5 to 15U per treatment of the pharmaceutical formulation of the present invention. In a further example, the subject receives between about 1.5 to 3U, 1.5 to 5U, 1.5 to 7U, 1.5 to 10U, 1.5 to 12U, 1.5 to 15U, 5 to 10U, 5 to 15U, or 10 to 15U per treatment are administered to a patient with hemifacial spasm. In a still further example, the subject receives about 1.5U, about 2U, about 2.5U, about 3U, about 3.5U, about 4U, about 4.5U about 5U, about 5.5U, about 6U, about 6.5U, about 7U, about 7.5U, about 8U, about 8.5U, about 9U, about 9.5U, about 10U, about 10.5U, about 11U, about 11.5U, about 12U, about 12.5U, about 13U, about 13.5U, about 14U, about 14.5U, or

about 15U per treatment are administered to a patient with hemifacial spasm. Dosages greater than 15U per treatment may also be administered to patients with hemifacial spasm to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0113] In an embodiment, the neuromuscular disease is cervical dystonia. A subject suffering from cervical dystonia, for example, receives between about 15 to 300U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 35 to 250U, 65 to 200U, 85 to 175U, 105 to 160U, or 125 to 145U are administered to a patient with cervical dystonia. In an embodiment, dosages to the sternocleidomastoid muscle are limited to 100U or less. Dosages greater than 300U per treatment may also be administered to patients with cervical dystonia to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0114] In an embodiment, the neuromuscular disease is blepharospasm. A subject suffering from blepharospasm, for example, receives between about 1.25 to 2.5U of a pharmaceutical formulation of the present invention injected into the medial and lateral pretarsal orbicularis oculi of the upper lid and into the lateral pretarsal orbicularis oculi of the lower lid. In a further example, the subject receives about 1.5U, about 1.6U, about 1.7U, about 1.8U, about 1.9U, about 2.0U, about 2.1 U, about 2.2U, about 2.3U, about 2.4U, about 2.5U, or more, per injection site. A treatment session can comprise multiple treatments.

[0115] In an embodiment, the neuromuscular disease is strabismus. A subject suffering from strabismus, for example, receives between about 1.25 to 2.5U per injection site of a pharmaceutical formulation of the present invention. In a further example, the subject receives about 1.5U, , about 1.6U, about 1.7U, about 1.8U, about 1.9U, about 2.0U, about 2.1U, about 2.2U, about 2.3U, about 2.4U, about 2.5U, or more, per injection site to achieve a therapeutic response. In embodiments, lower doses are used for treatment of small deviations. In embodiments, vertical muscles and horizontal strabismus of less than 20 prism diameters can be treated with 1.25 to 2.5U per injection site. A treatment session can comprise multiple treatments.

[0116] In an embodiment, the neuromuscular disease is muscle spasticity. A subject suffering from muscle spasticity, for example, receives between about 20 to 200U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 20 to 30U, 20 to 40U, 20 to 60U, 20 to 80U, 20 to 100U, 20 to 125U, 20 to 150U, or 20 to 175U per treatment are administered to a patient with muscle spasticity. In a still further example, the subject receives about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, about 150U, about 155U, about 160U, about 165U, about 170U, about 175U, about 180U, about 185U, about 190U, about 195U, or about 200U per treatment are administered to a patient with muscle spasticity. In an embodiment, the biceps brachii can be injected with between 100U and 200U divided into 4 injection sites. In an embodiment, the flexor carpi radialis can be injected with between 12.5U and 50U in 1 injection site. In an embodiment, the flexor carpi ulnaris can be injected with between 12.5U and 50U in 1 injection site. In an embodiment, the flexor digitorum profundus can be injected with between 30U and 50U in one injection site. In an embodiment, the flexor digitorum sublimis can be injected with between 30U and 50 in a single injection site. Dosages greater than 200U per treatment may also be administered to patients with muscle spasticity to achieve a therapeutic response. A treatment session can comprise multiple treatments.

Treatment of pain

[0117] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0118] In another embodiment, the present disclosure provides compositions for use in methods for treating pain comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce pain. In another embodiment, the patient suffers from myofascial pain, migraine headache pain, tension headache pain, neuropathic pain, facial pain, lower-back pain, sinus headache pain, pain associated with temporomandibular joint disease, pain associated with spasticity or cervical dystonia, post-surgical wound pain, or neuralgia. A treatment session can comprise multiple treatments.

[0119] In an embodiment, the patient suffers from facial pain. A subject suffering from facial pain, for example, receives between about 4 to 40U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 4 to 10U, 4 to 15U, 4 to 20U, 4 to 25U, 4 to 30U, 4 to 35U, 7 to 15U, 7 to 20U, 7 to 25U, 7 to 30U, 7 to 35U, or 7 to 40U per treatment are administered to a patient suffering from facial pain. In a still further example, the subject receives about 4U, about 5U, about 7.5U, about 10U, about 12.5U, about 15U, about 17.5U, about 20.0U, about 22.5U, about 25.0U, about 27.5U, about 30.0U, about 32.5U, about 35U, about 37.5U, or about 40U per treatment are administered to a patient with facial pain. Dosages greater than 40U per treatment may also be administered to patients with facial pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0120] In an embodiment, the patient suffers from myofascial pain. A subject suffering from myofascial pain, for example,

receives between about 5 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 5 to 10U, 5 to 20U, 5 to 30U, 5 to 40 Units, 5 to 50 Units, 5 to 60 Units, 5 to 70 Units, 5 to 80 Units, 5 to 90U, 10 to 20U, 10 to 30U, 10 to 50U, or 10 to 60U, or 10 to 70U, or 10 to 80U, 10 to 90U, or 10 to 100U per treatment are administered to a patient suffering from myofascial pain. In a further example, the subject receives about 5U, about 10U, about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, or about 100U per treatment are administered to a patient with myofascial pain. Dosages greater than 100U per treatment may also be administered to patients with myofascial pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0121] In an embodiment, the subject suffers from lower-back pain. A subject suffering from lower-back pain, for example, receives between about 15 to 150U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 15 to 30U, 15 to 50U, 15 to 75U, 15 to 100U, 15 to 125U, 15 to 150U, 20 to 100U, 20 to 150U, or 100 to 150U per treatment are administered to a patient with lower-back pain. In a still further example, the subject receives about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, or about 150U per treatment are administered to a patient with lower-back pain. Dosages greater than 150U per treatment may also be administered to patients with lower-back pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0122] In an embodiment, the patient suffers from migraine headache pain, including wherein the patient suffers from migraine headaches of 4 hours or more 15 or more days per month. A subject suffering from migraine-headache pain, for example, receives between about 0.5 to 200U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 5 to 190U, 15 to 180U, 25 to 170U, 35 to 160U, 45 to 150U, 55 to 140U, 65 to 130U, 75 to 120U, 85 to 110U, or 95 to 105U per treatment are administered to a patient suffering from migraine-headache pain. A treatment session can comprise multiple treatments.

[0123] For example, about 0.5U, about 1.0U, about 1.5U, about 2.0U, about 2.5U, about 3.0U, about 3.5U, about 4.0U, about 4.5U, about 5.0U, about 5.5U, about 6.0U, about 6.5U, about 7.0U, about 7.5U, about 8.0U, about 8.5U, about 9.0U, about 9.5U, about 10.0U, about 12U, about 15U, about 17U, about 20U, about 22U, about 25U, about 27U, about 30U, about 32U, about 35U, about 37U, about 40U, about 42U, about 45U, about 47U, or about 50U per treatment site are administered to a patient with migraine-headache pain. A patient can be treated at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, or more, or the like. In an embodiment, a patient suffering from migraine is injected 31 times with 5U per 0.1mL injection, across the corrugator (2 injections of 5U each), procerus (1 injection of 5U), frontalis (4 injections of 5U each), temporalis (8 injections of 5U each), occipitalis (6 injections of 5U each), cervical paraspinal (4 injections of 5U each), and trapezius (6 injections of 5U each) muscles. With the exception of the procerus muscle which can be injected at the midline, all muscles can, in certain embodiments, be injected bilaterally with half of the injection sites to the left and half to the right side of the head and neck. Dosages greater than 200U per treatment may also be administered to patients with migraine-headache pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0124] In an embodiment, the patient suffers from sinus-headache pain. A subject suffering from sinus-headache pain, for example, receives between about 4 to 40U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 4 to 10U, 4 to 15U, 4 to 20U, 4 to 25U, 4 to 30U, 4 to 35U, 7 to 15U, 7 to 20U, 7 to 25U, 7 to 30U, 7 to 35U, or 7 to 40U per treatment are administered to a patient suffering from sinus-headache pain. In a still further example, the subject receives about 4U, about 5U, about 7.5U, about 10U, about 12.5U, about 15U, about 17.5U, about 20.0U, about 22.5U, about 25.0U, about 27.5U, about 30.0U, about 32.5U, about 35U, about 37.5U, or about 40U per treatment are administered to a patient with sinus-headache pain. Dosages greater than 40U per treatment may also be administered to patients with sinus headache-pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0125] In an embodiment, the patient suffers from tension-headache pain. A subject suffering from tension-headache pain, for example, receives between about 5 to 50U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 5 to 10U, 5 to 15U, 5 to 20U, 5 to 25U, 5 to 30U, 5 to 35U, 5 to 40U, 5 to 45U, 10 to 20U, 10 to 25U, 10 to 30U, 10 to 35U, 10 to 40U, or 10 to 45U per treatment are administered to a patient with tension-headache pain. In a still further example, the subject receives about 5U, about 10U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, or about 50U per treatment are administered to a patient with tension-headache pain. In an embodiment, a patient suffering from tension headache is injected 31 times with 5U per 0.1mL injection, across the corrugator (2 injections of 5U each), procerus (1 injection of 5U), frontalis (4 injections of 5U each), temporalis (8 injections of 5U each), occipitalis (6 injections of 5U each), cervical paraspinal (4 injections of 5U

each), and trapezius (6 injections of 5U each) muscles. With the exception of the procerus muscle which can be injected at the midline, all muscles can, in certain embodiments, be injected bilaterally with half of the injection sites to the left and half to the right side of the head and neck. Dosages greater than 200U per treatment may also be administered to patients with tension headache pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0126] In an embodiment, the patient suffers from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis. For example a pharmaceutical formulation of the present invention can be administered to the nasal mucosa or to the subcutaneous structures overlying the sinuses, wherein the administration of the formulation reduces the headache and/or facial pain associated with acute recurrent or chronic sinusitis. In further embodiments, any of the pharmaceutical formulations of the present invention can be administered to the nasal mucosa or to the subcutaneous structures overlying the sinuses, such as over one or more of the sinuses selected from the group consisting of: ethmoid; maxillary; mastoid; frontal; and sphenoid. In another embodiment, subcutaneous structures overlying the sinuses lie within one or more of the areas selected from the group consisting of: forehead; malar; temporal; post auricular; and lip. In embodiments, multiple injections of 5U each are administered to treat the sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis.

[0127] In another embodiment, a patient suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis is treated by administering any of the pharmaceutical formulations of the present invention to an afflicted area of the patient. In a further embodiment, the pharmaceutical formulations disclosed herein are administered to the projections of a trigeminal nerve innervating a sinus.

[0128] Patients suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis often exhibit symptoms including rhinitis, sinus hypersecretion and/or purulent nasal discharge. In one embodiment, patients treated with the pharmaceutical formulations of the present invention exhibit symptoms of sinus hypersecretion and purulent nasal discharge.

[0129] Embodiments of the present invention also provide compositions for use in methods for treating a patient suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis, wherein the subject suffers from neuralgia. In certain embodiments the neuralgia is trigeminal neuralgia. In another embodiment, the neuralgia is: associated with compressive forces on a sensory nerve; associated with intrinsic nerve damage, demyelinating disease, or a genetic disorder; associated with a metabolic disorder; associated with central neurologic vascular disease; or associated with trauma. In another embodiment of the present invention, the pain is associated with dental extraction or reconstruction.

Treatment of urological disorders

[0130] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0131] In an embodiment, the invention also provide compositions for use in methods for treating a patient suffering from overactive bladder (OAB), such as, for example, that due to a neurologic condition (NOAB), or idiopathic OAB (IOAB). For example, pharmaceutical formulations of the present invention can be administered to the bladder or its vicinity, e.g. the detrusor, wherein the administration of the formulation reduces the urge incontinence associated with overactive bladder. In certain embodiments, the dosage can be, for example, 200U, or more, or less, or the like. For example, the dosage can be about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, about 150U, about 160U, about 170U, about 180U, about 190U, about 200U, about 210U, about 220, about 230U, about 240U, or more, or the like, per treatment. A patient can be injected at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, 33 sites, 34 sites, 35 sites, 36 sites, 37 sites, 38 sites, or more, or the like. In an embodiment, patients suffering from OAB are treated with 30 mL injections of approximately 6.7U per injection into the detrusor muscle.

[0132] In an embodiment, the invention also provides compositions for use in methods for treating a patient suffering from neurogenic detrusor overactivity (NDO), such as that due to a neurologic condition. For example, pharmaceutical formulations of the present invention can be administered to the bladder or its vicinity, e.g. the detrusor, wherein the administration of the formulation reduces the urge incontinence associated with overactive bladder. In certain embodiments, the dosage can be, for example, 200U, or more, or less, or the like. For example, the dosage can be about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, about 150U, about 160U, about 170U, about 180U, about 190U, about 200U, about 210U, about 220, about 230U, about 240U, or more, or the like, per

treatment. A patient can be injected at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, or more, or the like. In an embodiment, patients suffering from NDO are treated with 30 1mL injections of approximately 6.7U per injection into the detrusor muscle.

Treatment of cosmetic features

[0133] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0134] In another embodiment, the present invention provides methods for cosmetically modifying soft-tissue features comprising the step of administering at least one pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to modify said features. In a further embodiment, the pharmaceutical formulation is administered via transcutaneous or transmucosal injection either at a single focus or multiple foci.

[0135] In embodiments, pharmaceutical formulations of the present invention are administered to the face or neck of the subject. In a further embodiment, the pharmaceutical formulations of the present invention are administered to the subject in an amount sufficient to reduce rhytides. For example, the formulation can be administered between eyebrows of the subject in an amount sufficient to reduce vertical lines between the eyebrows and on a bridge of a nose. The pharmaceutical formulations can also be administered near either one or both eyes of the subject in an amount sufficient to reduce lines at corners of the eyes. In an embodiment, compositions of the invention can be injected locally to smooth skin. In another embodiment, the pharmaceutical formulations of the present invention can also be administered to a forehead of the subject in an amount sufficient to reduce horizontal lines on said forehead. In yet another embodiment of the present invention the pharmaceutical formulation is administered to the neck of the subject in an amount sufficient to reduce muscle bands in the neck. In an embodiment, a pharmaceutical composition is applied to the masseter muscle to relax the muscle and / or decrease masseter mass.

[0136] In a further embodiment, the patient suffers from facial wrinkles. A subject suffering from facial wrinkles, for example, can receive between about 1 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 1 to 10U, 1 to 20U, 1 to 30U, 1 to 40U, 1 to 50U, 1 to 60U, 1 to 70U, 1 to 80U, 1 to 90U, 5 to 20U, 5 to 30U, 5 to 40U, 5 to 50U, 5 to 60U, 5 to 70U, 5 to 80U, 5 to 90U, or 5 to 100U per treatment are administered to a patient with an inflammatory disorder. In a still further example, about 1U, about 10U, about 20U, about 30U, about 40U, about 50U, about 60U, about 70U, about 80U, about 90U, or about 100U per treatment are administered to a patient. Dosages greater than 100U per treatment may also be administered to patients suffering from inflammation or an inflammatory disorder to achieve a therapeutic response.

Treatment of inflammation

[0137] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0138] In another embodiment, the present invention provides compositions for use in methods for treating inflammation comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce inflammation. In certain embodiments, pharmaceutical formulations of the present invention are administered to a patient without producing muscle weakness. In an embodiment, the pharmaceutical formulations of the present invention are administered to patients with an inflammatory condition. In certain embodiments the inflammatory condition is neurogenic inflammation. In another embodiment, the subject suffers from rheumatoid arthritis or a gastro-intestinal inflammatory disease.

[0139] In a further embodiment, the patient suffers from an inflammatory disorder. A subject suffering from an inflammatory disorder, for example, receives between about 1 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 1 to 10U, 1 to 20U, 1 to 30U, 1 to 40U, 1 to 50U, 1 to 60U, 1 to 70U, 1 to 80U, 1 to 90U, 5 to 20U, 5 to 30U, 5 to 40U, 5 to 50U, 5 to 60U, 5 to 70U, 5 to 80U, 5 to 90U, or 5 to 100U per treatment are administered to a patient with an inflammatory disorder. In a still further example, about 1U, about 10U, about 20U, about 30U, about 40U, about 50U, about 60U, about 70U, about 80U, about 90U, or about 100U per treatment are administered to a patient. Dosages greater than 100U per treatment may also be administered to patients suffering from inflammation or an inflammatory disorder to achieve a therapeutic response.

Treatment of skin conditions

[0140] The following serves to illustrate a use of the pharmaceutical composition manufactured according to the method of the invention.

[0141] A composition for use in a method within the scope of the present invention for treating a skin disorder can have the step of local administration of a botulinum neurotoxin to a location of a skin disorder of a patient, such as to a face, hand or foot of a patient. The neurotoxin can be locally administered in an amount of between about 10-3 units/kg of patient weight and about 35 units/kg of patient weight. For example, the neurotoxin is locally administered in an amount of between about 10-2 U/kg and about 25 U/kg of patient weight. In a further example, the neurotoxin is administered in an amount of between about 10-1 U/kg and about 15 U/kg. In one composition for use in a method within the scope of the present invention, the neurotoxin is locally administered in an amount of between about 1 U/kg and about 10 U/kg. In a clinical setting it can be advantageous to administer from 1U to 3000U of a neurotoxin, such as botulinum toxin type A or B, to a skin disorder location by topical application or by subdermal administration, to effectively treat the skin disorder.

[0142] Administration of botulinum toxin can be carried out at multiple sites in the skin, wherein the sites of adjacent injections are separated by about 0.1 to 10cm, or about 0.5 to about 5cm, for example, by about 1.5 to about 3cm. The toxins may be any of the botulinum toxins A, B, C, D, E, F or G. The amounts administered may vary between 0.1 and 1000U, or about 1 to about 40, or from about 5 to about 10U, depending on the manufactures specifications, the class of the toxin and the mode of administration. The repeat time range for these administrations for maintenance of the desired change varies substantially according to the location of the injection, the condition to be adjusted and the condition of the patient. Thus the repeat time may vary from about 1 week to about 50 weeks, however a common range is about 4 to about 25 weeks, or even about 12 weeks to about 16 weeks.

[0143] The distances between administrations, for example, injections, can vary from about 1 mm to about 10cm, suitably from about 5mm to about 5cm, and more usually from about 1cm to about 3cm. Thus for example botulinum A may be suitably administered by intradermal injection between about 0.1 to about 10U at a separation of from about 0.5 to about 10cm.

[0144] In another embodiment, the present invention provides compositions for use in methods for treating cutaneous disorders comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce a sebaceous or mucous secretion. In further embodiments, the pharmaceutical formulations of the present invention are administered to a patient without producing muscle weakness. In certain embodiments the pharmaceutical formulations of the present invention are injected into one or more sites of an eyelid or conjunctiva. In another embodiment, the formulations of the present invention are administered to a body surface.

[0145] In another embodiment, the pharmaceutical formulations are administered in an amount sufficient to reduce cutaneous bacterial or fungal growth, including but not limited to Staphylococcus; Streptococcus and Moraxella. For example, the pharmaceutical formulations of the present invention are administered to an area selected from the group consisting of: eyelid; scalp; feet; groin; and armpit to reduce cutaneous infection.

EXAMPLES

Reference Example 1

ACTIVITIES AND STABILITIES OF EXEMPLARY SOLID PHARMACEUTICAL COMPOSITIONS RELATIVE TO PRIOR ART FORMULATIONS.

[0146] Bulk solutions of botulinum toxin were prepared by mixing an appropriate aliquot of a botulinum toxin type A with several vehicle solutions as described in the following Tables 1-3. The solutions were filled into glass vials and lyophilized using conventional freeze-drying conditions. Potency of the lyophilized formulations was tested by a cell based potency assay (CBPA) after reconstitution of the lyophiles with saline. Potency recovery results after freeze-drying and after storage at indicated temperatures are provided in the tables and normalized to target potency. The potencies of the solid compositions prepared according to aspects of the present invention were compared to three comparative formulations as shown in Tables 1-3.

Table 1.1: Lyophilized formulations

Formulation	Excipient, % w/w						Buffer	Normalized potency, storage at 25 °C		
	Treh	TWEEN® 20	P 188	NaCl	Met	NAC		T0	3 mo	6 mo
Comparator 1	3	0.04		0.9	0.2	-	water	80.6%	86.4%	80.8%
Comparator 2	2	-	4	-	-	-	20mM Histidine pH 5.5	81.5%	68.6%	68.0%
Formulation 1*	2	-	4	-	-	0.03	20mM Histidine pH 5.5	98.6%	91.9%	86.9%
Treh = trehalose; P 188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine. *this formulation does not form part of the invention and is provided for information only										

Table 1.2: Lyophilized formulations

Formulation	Excipient, % w/w						Buffer	Normalized potency, storage at 40 °C		
	Treh	TWEEN® 20	P 188	NaCl	Met	NAC		1 mo	3 mo	6 mo
Comparator 1	3	0.04		0.9	0.2		Water	74.8%	70.3%	24.0%
Comparator 2	2		4				20mM Histidine pH 5.5	64.8%	57.8%	46.8%
Formulation 1*	2		4			0.03	20mM Histidine pH 5.5	86.5%	77.9%	62.3%
Treh = trehalose; P188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine. *this formulation does not form part of the invention and is provided for information only										

Table 2: Lyophilized formulations

Formulation	Excipient, % w/w							Buffer	Normalized potency (% of target)		
	Treh	Sucr	TWEEN® 20	P 188	NaCl	Met	NAC		T0	1 mo -20 °C	1 mo 40 °C
Comparator 2	2			4				20mM Histidine pH 5.5	87.15%	88.95%	76.32%
Comparator 3		3	0.04		0.9	0.2		Water	84.06%	85.18%	72.86%
Formulation 2*	2			4		0.2		20mM Histidine pH 6.0	98.60%	120.85%	91.17%
Formulation 3*	8		0.04				0.03	20mM Histidine pH 6.0	105.2%	110.05%	96.89%
Treh = trehalose; Sucr = sucrose; P188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine. *this formulation does not form part of the invention and is provided for information only											

Table 3: Lyophilized formulations

Formulation	Excipient, % w/w						Buffer	Normalized potency, storage at 25 °C			
	Treh	Sucr	TWEEN® 20	P188	NaCl	Met		NAC	T0	3 mo	7.5 mo
Comparator 2	2			4				20mM Histidine, pH 5.5	87.15%	78.0%	78.0%
Formulation 2	2			4		0.2		20mM Histidine, pH 6.0	98.6%	97.0%	98.0%
Formulation 4	8			0.6		0.2		20mM Histidine, pH 6.0	86.48%	83.0%	84.0%
P-188 = Poloxamer P-188; Met = L-methionine											

Example 2

ACTIVITIES OF LIQUID PHARMACEUTICAL COMPOSITIONS IN THE PRESENCE OR ABSENCE OF ANTIOXIDANTS

[0147] Bulk drug product solutions were prepared by mixing an appropriate aliquot of a botulinum toxin type A with three different vehicle solutions as shown in Table 4. All three formulations contained 8% w/w trehalose, 4% w/w P188 and 20 mM Histidine buffer at pH 6.0. The target potency was 100units/mL. Formulation 10 contained no antioxidant. Formulations 11 and 12 contained NAC and methionine, respectively. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by a cell based potency assay (CBPA) after filling (time zero, t₀) and storage for one month at four temperatures (-70, 5, 25, and 40°C). Potency test results are given in Table 4. Briefly, methionine-containing formulation (#12) retained its potency after one month storage at all four temperatures, including 40°C, whereas the formulation w/o an antioxidant lost approx. 17 % potency at 25°C and lost essentially all activity (i.e., complete inactivation) at 40°C. A second antioxidant tested, N-acetyl-L-cysteine, accelerated loss of potency as compared with the antioxidant-free formulation, demonstrating that N-acetyl-L-cysteine acted as pro-oxidant in this formulation. The liquid composition comprising NAC lost over 50% potency after storage for 1 month at 25 °C and lost essentially all activity (i.e., complete inactivation of the toxin) after storage for 1 month at 40 °C. In contrast, as shown in Table 1, the lyophilized compositions comprising NAC lost 7% ((98.6%-91.9%)/98.6%) potency after storage for 3 months at 25 °C and lost 12% ((98.6%-86.5%)/98.6%) potency after storage for 1 month at 40 °C. This demonstrates that NAC can function as a stabilizer in the lyophilized compositions.

Table 4: Liquid formulations

Formulation No.*	Antioxidant	Potency, U/mL				
		T0	1 mo. -70°C	1 mo. 5°C	1 mo. 25°C	1 mo. 40°C
Formulation 10**	none	128	135	135	106	0.225
Formulation 11**	N-acetyl-L-cysteine, 0.2 % w/w	128	133	129	61	0.2
Formulation 12	L-methionine, 0.2% w/w	133	146	146	145	138

*Each formulation contained the same amount of botulinum toxin, 8 w/w% trehalose, and 4 w/w% poloxamer P188 in histidine buffer.

**this formulation does not form part of the invention and is provided for information only

Example 3

IMPACT OF EXEMPLARY ANTIOXIDANTS ON THE STABILITY OF EXEMPLARY LIQUID FORMULATIONS.

[0148] Bulk drug product solutions were prepared by mixing an appropriate aliquot of a botulinum toxin type A with different antioxidants as shown in Table 5. All formulations contained 8% w/w trehalose, 4% w/w P188, 20 mM Histidine buffer at pH 6.0 and one or more antioxidants as listed. Target potency was 100 U/mL. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by a cell based potency assay (CBPA) after filling (time zero, t₀) and storage for two weeks and 1 month at 40°C. Potency test results are given in Table 5. Liquid compositions comprising NAC and EDTA sodium salt (Formulation 26) retained full potency after storage at 40 °C for 2 weeks and 1 month, as did a composition comprising NAC, EDTA sodium salt and tryptophan (Formulation 27). In contrast, as shown in Table 5, liquid compositions comprising NAC but not EDTA (Formulation 25) lost essentially all potency after 2 weeks storage at 40 °C. This demonstrates that the combination of antioxidants - a chelating agent (e.g., EDTA, EGTA or DTPA) and/or a chain terminator antioxidant (e.g., methionine, cysteine, NAC and BHT) provides a stabilizing effect on the botulinum toxin.

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Table 5: Liquid formulations

Formulation No. ¹	Antioxidant ²							Potency U/mL		
	NAC %	Met %	TRP %	GSH %	NaSul %	PrpGal %	EDTA %	T0	2 wks 40 °C	1 mo. 40 °C
Formulation 20		0.2						126	129	130
Formulation 21*			0.2					127	13.71	NT ³
Formulation 22*				0.2				123	3.76	NT
Formulation 23*					0.2			23.7	0.161	NT
Formulation 24*						0.2		0.164	0.150	NT
Formulation 25*	0.2		0.2					133	0.253	NT
Formulation 26*	0.2						0.03	129	127	127
Formulation 27*	0.2		0.2				0.03	129	125	122
Formulation 28*			0.2	0.2				126	2.45	NT
¹ Each formulation contained 100 U/mL botulinum toxin, 8 w/w% trehalose, and 4 w/w% poloxamer P188 in 20 mM histidine buffer, pH 6.0 and the specified antioxidant. ² NAC = N-acetyl-L-cysteine; Met = L-methionine; TRP = L-tryptophan; GSH = L-glutathione; NaSul = sodium sulfite; PrpGal = propyl gallate; EDTA = ethylene diamine tetraacetic acid, sodium salt. ³ NT=not tested *these formulations do not form part of the invention and are provided for information only										

Example 4

IMPACT OF CHOICE OF SURFACTANT ON THE STABILITY OF EXEMPLARY LIQUID COMPOSITIONS

[0149] Bulk drug product solutions were prepared by mixing an appropriate aliquot of a botulinum toxin type A with a poloxamer or a polysorbate as shown in Table 6. All formulations contained 8% w/w trehalose, 0.2% w/w methionine in 20 mM Histidine buffer at pH 6.0 and 4% w/w P188 or 0.04% w/w Tween-20 as listed. Target potency was 100 U/mL. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by a cell based potency assay (CBPA) after filling to give an initial potency. Potency was measured again after storage for 1 month at 40°C. Potency test results are given in Table 6. As is evident from the results in table 6, the liquid formulation comprising poloxamer provided improved stability after storage at 40°C for one month relative to the corresponding formulation comprising polysorbate.

Table 6: Liquid formulations

Formulation no.	Tre	Tw-20	P188	Met	Buffer	Storage time (Months)	Storage Temp.	CBPA (U/mL) Initial	CBPA (U/mL) 1 month
29	8	0	4	0.2	20 mM His pH 6.0	1	40°C	133	138

(continued)

Formulation no.	Tre	Tw-20	P188	Met	Buffer	Storage time (Months)	Storage Temp.	CBPA (U/mL) Initial	CBPA (U/mL) 1 month
30*	8	0.04	0	0.2	20 mM His, pH 6.0	1	40°C	114	9
Tre = Trehalose; P-188 = Poloxamer-188; Tw-20 = Tween-20; Met = L-Methionine; His = L-Histidine. CBPA gives the residual activity expressed in U/ml *this formulation does not form part of the invention and is provided for information only									

Example 5

IMPACT OF ABSENCE OF TONICITY AGENT ON THE STABILITY OF EXEMPLARY LIQUID COMPOSITIONS

[0150] Bulk drug product solutions were prepared by mixing an appropriate aliquot of a botulinum toxin type A with different components as shown in Tables 7 and 8. The amounts of components contained were 0% or 8% w/w trehalose, 0% or 0.2% w/w methionine, 0% or 4% w/w P188, all of which were formulated in 20 mM Histidine buffer at pH 6.0. Target potency was 100, 150, or 200 U/mL. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by a cell based potency assay (CBPA) after filling to give an initial potency. Potency was measured again after storage for 1 month at -70°C and 40°C. Potency test results are given in Tables 7 and 8. As is evident from the results in these tables, liquid formulations comprising poloxamer and no tonicity agent provide excellent stability after storage for one month at -70°C and 40°C. This is supported by the results in table 10 below for formulation 40. Meanwhile, liquid formulations comprising polysorbate and no tonicity agent provide some stability after storage for one month at -70°C.

Table 7: Liquid formulations

Formulation no.	Formulation composition (% w/v)				CBPA, U/mL	CBPA, U/mL	CBPA, U/mL	CBPA, U/mL
	trehalose	P188	Met	Buffer	target	Initial (U/ml)	1 month, -70°C	1 month, 40°C
31*	8	4	0	20 mM His pH 6.0	100	128	135	0.225
32*			0.2	20mM His pH 6.0	150	52	13	0
33*	8		0.2	20mM His pH 6.0	150	131	145	30
34*		4	0.2	20mM His pH 6.0	100	128	142	103
35	8	4	0.2	20mM His pH 6.0	100	126		130

Table 8: Liquid formulations

Formulation No.	Tre	Sucr	Tw-20	P188	Met	Buffer	Target potency (U/mL)	CBPA (U/ml) -70 °C	CBPA (U/ml) 40 °C
36*	0	0	0.04	0	0.2	20 mM His pH 6.0	100	25	1
37*	0	0	0	4	0.2	20 mM His pH 6.0	100	105	72

Sucr = sucrose; Tre = Trehalose; P-188 = Poloxamer-188; Tw-20 = Tween-20; Met = L-Methionine; His = L-Histidine.
 Initial = initial activity expressed in U/ml
 Results after 1 month are the residual activity expressed in U/ml
 *these formulations do not form part of the invention and are provided for information only

Example 6

STABILITY OF VARIOUS LIQUID COMPOSITIONS

[0151] Bulk drug product solutions were prepared by mixing an appropriate aliquot of a botulinum toxin type A with vehicle solutions, containing different stabilizers and surfactants as shown in Table 9. All formulations contained 8% or 0% w/w trehalose or sucrose, 4% w/w P188 or 0.04% w/w PS-20, optionally 20 mM Histidine buffer at pH 6.0, 0.2% w/w methionine, and optionally 0.9% w/w NaCl. Target potency was 100 U/mL. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by cell based potency assay (CBPA) after filling (time zero, t₀) and storage at 5°C. Potency test results are given in Table 10.

Table 9: Liquid formulations

Formulation No.	Target potency U/mL	Tre %	Suc %	NaCl %	P-188 %	PS-20 %	Met %	Buffer
38*	100		8		4		0.2	20 mM His, pH 6.0
39*	100		8		4		0.2	Water
40*	100			0.9	4		0.2	20 mM His, pH 6.0
41*	100	8				0.04	0.2	20 mM His, pH 6.0

Sucr = sucrose; Tre = Trehalose; P-188 = Poloxamer-188; Ps-20 = Tween-20; Met = L-Methionine; His = L-Histidine.
 *this formulation does not form part of the invention and is provided for information only

Table 10: Liquid formulations

Formulation No.	Storage Temperature	0 months	5.5 months	7.5 months	12 months
38*	5 °C	117	88	NT	94
39*	5 °C	114	NT	108	91
40*	5 °C	94	NT	80	62
41*	5 °C	114	NT	117	91
Results are the residual activity expressed in U/ml *this formulation does not form part of the invention and is provided for information only					

Reference Example 7

IMPACT OF CHOICE OF SUGAR ON STABILITY OF EXEMPLARY LYOPHILIZED FORMULATIONS

[0152] Bulk solutions of botulinum toxin were prepared by mixing an appropriate aliquot of a botulinum toxin type A with several vehicle solutions as described in Table 11. The amounts of components contained were 0% or 8% w/w trehalose or sucrose, 0.2% w/w methionine, 0% or 4% w/w P188, 0% or 0.04% w/w Tween 20 all of which were formulated in 20 mM Histidine buffer at pH 6.0. Target potency was 200 U/mL. These amounts are shown in Table 11 below. The solutions were filled into glass vials and lyophilized using conventional freeze-drying conditions. Potency of the lyophilized formulations was tested by a cell based potency assay (CBPA) after reconstitution of the lyophilized compositions after storage for 2 weeks at -70°C or 40°C. Potency recovery results are given in Table 12. By comparing Formulation No. 42 with Formulation No. 44 and Formulation No. 43 with Formulation No. 45, the impact of the type of sugar on these lyophilized formulations can be derived. The potency of the botulinum toxin in Formulation 42 and in Formulation 44 after 2 weeks of storage at -20 °C and at 40 °C reveals a higher potency recovery was achieved in lyophilized compositions comprising trehalose, as compared to lyophilized compositions comprising sucrose.

Table 11: Lyophilized formulations

Formulation No.	Tre	Sucr	Tw-20	P188	Met	Buffer	Target potency (U/vial)
42*	8	0	0	4	0.2	20 mM His pH 6.0	200
43*	8	0	0.04	0	0.2	20 mM His pH 6.0	200
44*	0	8	0	4	0.2	20 mM His pH 6.0	200
45*	0	8	0.04	0	0.2	20 mM His pH 6.0	200
Tre = Trehalose; Sucr = Sucrose; Tw-20 = Tween-20; P-188 = Poloxamer-188; Met = L-Methionine; His = L-Histidine. *these formulations do not form part of the invention and are provided for information only							

Table 12: Lyophilized formulations

Formulation No.	-20 °C, 2 Weeks	40 °C, 2 Weeks
42*	166	162
43*	147	137
44*	149	134
45*	150	142
*these formulations do not form part of the invention and are provided for information only		

Reference Example 8

IMPACT OF CHOICE OF SURFACTANT ON THE STABILITY OF EXEMPLARY LYOPHILIZED COMPOSITIONS

[0153] The results in Tables 11 and 12 also demonstrate the impact of the choice of surfactant on the stability of certain lyophilized compositions. By comparing Formulation No. 42 with Formulation No. 43 and Formulation No. 44 with Formulation No. 45, the impact of the type of surfactant on these lyophilized formulations can be derived. The potency of the botulinum toxin in Formulation 43 and in Formulation 45 after 2 weeks of storage at -20 °C and at 40 °C reveals a higher potency recovery was achieved in lyophilized compositions comprising a poloxamer surfactant, as compared to lyophilized compositions comprising a polysorbate surfactant.

Reference Example 9

TREATMENT OF DEPRESSION WITH EXEMPLARY COMPOSITIONS

[0154] A 58-year-old college professor presents symptoms of depression. Her physician diagnoses recurrent moderate to severe major depressive disorder (MDD) based upon the DSM-IV-TR criteria. The physician administers by intramuscular injection a botulinum toxin solution made using the composition shown in Table A reconstituted in saline into the procerus and corrugator muscles in a single treatment session. 30 units in total are administered at 6 injection sites. The procerus and corrugator muscles are paralysed. It is later confirmed using the Montgomery-Asberg Depression Rating scale that the treatment has alleviated the depression. Additional efficacy measures including the clinic CGI-S score (Clinical Global Impression of Change scores), and the clinic HAM-D17 total score (Hamilton Rating Scale for Depression) further confirm this. The treatment is repeated after 3 months.

Reference Example 10

TREATMENT OF DEPRESSION WITH EXEMPLARY COMPOSITIONS

[0155] A 21-year-old student presents symptoms of depression. His physician diagnoses single episode moderate to severe major depressive disorder (MDD) based upon the DSM-IV-TR criteria. The physician administers by intramuscular injection a botulinum toxin solution made using the composition shown in Table A reconstituted in saline to the procerus and corrugator muscles in a single treatment session. 50 units in total are administered at 8 injection sites. The procerus and corrugator muscles are paralysed. It is later confirmed using the Montgomery-Asberg Depression Rating scale that the treatment has alleviated the depression. Additional efficacy measures including the clinic CGI-S score (Clinical Global Impression of Change scores), and the clinic HAM-D17 total score (Hamilton Rating Scale for Depression) further confirm this. The treatment is repeated after 6 months.

Reference Example 11

TREATMENT OF CARDIAC ARRHYTHMIA WITH EXEMPLARY COMPOSITIONS

[0156] A 41-year-old teacher undergoing cardiac surgery shows symptoms of cardiac arrhythmia. The surgeon administers a botulinum toxin solution made using the composition shown in Table A reconstituted in saline into the major epicardial fat pads of the heart of the patient. 50 U is administered per epicardial fat pad, up to a total of 250 U. The patient is followed by ECG for a four week period following the injection. The incidence of atrial fibrillation is reduced. In addition, the following improvements are noted for this patient: the length of hospital stay is reduced, length of stay in ICU is reduced, anticoagulant medication use is reduced, and the need for interventional procedures for Post Operative Atrial fibrillation(POAF) such as ablation, pacemaker implantation, electrical or pharmacologic cardioversion are reduced.

Reference Example 12: treatment of cardiac arrhythmia with exemplary compositions.

[0157] A 66-year-old pensioner undergoing cardiac surgery shows symptoms of cardiac arrhythmia. The surgeon administers a botulinum toxin solution made using the composition shown in Table A reconstituted in saline into the major epicardial fat pads of the heart of the patient. 25 U is administered per epicardial fat pad, up to a total of 125 U. The patient is followed by ECG for a four week period following the injection. The incidence of atrial fibrillation is reduced. In addition, the following improvements are noted for this patient: the length of hospital stay is reduced, length of stay in ICU is reduced, anticoagulant medication use is reduced, and the need for interventional procedures for Post Operative Atrial fibrillation(POAF) such as ablation, pacemaker implantation, electrical or pharmacologic cardioversion are reduced.

[0158] Many alterations and modifications may be made by those having ordinary skill in the art, without departing from the scope of the disclosure.

5 Claims

1. A method of manufacturing a liquid pharmaceutical composition without a lyophilisation process, wherein the liquid pharmaceutical composition comprises:

- 10 (i) a botulinum toxin;
 (ii) poloxamer 188;
 (iii) methionine; and
 (iv) trehalose
 wherein the composition comprises a buffer, wherein the buffer is histidine, and wherein the pH is in the range 5-7,
 15 wherein the relative weight amounts (% w/w) of trehalose, poloxamer 188, methionine are within the following ranges:

20	trehalose	1 to 10
	poloxamer 188	0.5 to 5
	methionine	0.1 to 0.3.

- 25 2. The method of manufacturing a liquid pharmaceutical composition according to claim 1, wherein the liquid pharmaceutical composition further comprises NaCl and/or EDTA, wherein the relative weight amounts (% w/w) of NaCl and EDTA are optionally within the following ranges:

30	NaCl	0.1 to 10 and/or
	EDTA	0.01 to 0.1.

- 35 3. The method of manufacturing a liquid pharmaceutical composition according to claim 1, wherein the relative weight amounts (% w/w) of trehalose, poloxamer 188, methionine, and optionally NaCl, and optionally EDTA in the liquid pharmaceutical composition are:

a)

40	trehalose-	7 to 9
	poloxamer 188	3.5 to 4.5
	methionine	0.15 to 0.25;

and are preferably

45	trehalose	8
	poloxamer 188	4
	methionine	0.2; or

50 b)

55	trehalose	7 to 9
	poloxamer 188	0.5 to 0.7
	methionine	0.15 to 0.25;

and are preferably

trehalose	8
poloxamer 188	0.6
methionine	0.2; or

c)

trehalose	1 to 3
poloxamer 188	3.5 to 4.5
methionine	0.15 to 0.25
NaCl	0.4 to 0.8;

and are preferably

trehalose	2
poloxamer 188	4
methionine	0.2
NaCl	0.6; or

d)

trehalose	7 to 9
poloxamer 188	3.5 to 4.5
methionine	0.15 to 0.25
EDTA	0.01 to 0.05;

and are preferably

trehalose	8
poloxamer 188	4
methionine	0.2
EDTA	0.03.

4. The method of manufacturing a liquid pharmaceutical composition according to any one of claims 1 - 3, wherein in the liquid pharmaceutical composition the concentration of histidine is 20 mM, and wherein in the liquid pharmaceutical composition the pH is preferably in the range 5.5-6.
5. The method of manufacturing a liquid pharmaceutical composition according to any one of claims 1 to 4, wherein the liquid pharmaceutical composition consists of one or more botulinum toxins, trehalose, poloxamer 188, methionine, a buffer wherein the buffer is histidine, and optionally NaCl, and optionally EDTA
6. The method of manufacturing a liquid pharmaceutical composition according to any previous claim, wherein the liquid pharmaceutical composition comprises no animalderived protein.
7. The method of manufacturing a liquid pharmaceutical composition according to any one of claims 1 to 6, wherein the liquid pharmaceutical composition comprises ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog, wherein the relative weight amount (% , w/w) of EDTA optionally ranges from about 0.01 to 0.10.

Patentansprüche

1. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung ohne Lyophilisierungsverfahren, bei dem die flüssige pharmazeutische Zusammensetzung folgendes umfasst:

- (i) ein Botulinumtoxin;
- (ii) Poloxamer 188;
- (iii) Methionin; und
- (iv) Trehalose,

wobei die Zusammensetzung einen Puffer umfasst, wobei der Puffer Histidin ist und wobei der pH-Wert im Bereich von 5-7 liegt,
wobei die relativen Gewichtsmengen (% w/w) an Trehalose, Poloxamer 188 und Methionin innerhalb der folgenden Bereiche liegen:

Trehalose	1 bis 10
Poloxamer 188	0,5 bis 5
Methionin	0,1 bis 0,3.

2. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß Anspruch 1, bei dem die flüssige pharmazeutische Zusammensetzung ferner NaCl und/oder EDTA umfasst, wobei die relativen Gewichtsmengen (% w/w) an NaCl und EDTA optional innerhalb der folgenden Bereiche liegen:

NaCl	0,1 bis 10 und/oder
EDTA	0,01 bis 0,1.

3. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß Anspruch 1, bei dem die relativen Gewichtsmengen (% w/w) an Trehalose, Poloxamer 188, Methionin, und optional NaCl, und optional EDTA in der flüssigen pharmazeutischen Zusammensetzung die folgenden sind:

a)

Trehalose	7 bis 9
Poloxamer 188	3,5 bis 4,5
Methionin	0,15 bis 0,25;

und bevorzugt

Trehalose	8
Poloxamer 188	4
Methionin	0,2; oder

b)

Trehalose	7 bis 9
Poloxamer 188	0,5 bis 0,7
Methionin	0,15 bis 0,25;

und bevorzugt

Trehalose	8
Poloxamer 188	0,6
Methionin	0,2; oder

c)

Trehalose	1 bis 3
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(fortgesetzt)

Poloxamer 188	3,5 bis 4,5
Methionin	0,15 bis 0,25
NaCl	0,4 bis 0,8;

und bevorzugt

Trehalose	2
Poloxamer 188	4
Methionin	0,2
NaCl	0,6; oder

d)

Trehalose	7 bis 9
Poloxamer 188	3,5 bis 4,5
Methionin	0,15 bis 0,25
EDTA	0,01 bis 0,05;

und bevorzugt

Trehalose	8
Poloxamer 188	4
Methionin	0,2
EDTA	0,03.

4. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß mindestens einem der Ansprüche 1-3, bei dem die Konzentration an Histidin in der flüssigen pharmazeutischen Zusammensetzung 20 mM beträgt, und bei dem der pH Wert in der flüssigen pharmazeutischen Zusammensetzung vorzugsweise im Bereich von 5,5-6 liegt.
5. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß mindestens einem der Ansprüche 1 bis 4, wobei die flüssige pharmazeutische Zusammensetzung aus ein oder mehr Botulinumtoxinen, Trehalose, Poloxamer 188, Methionin, einem Puffer, wobei der Puffer Histidin ist, und optional NaCl, und optional EDTA besteht.
6. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß mindestens einem der vorhergehenden Ansprüche, bei dem die flüssige pharmazeutische Zusammensetzung kein tierisches Protein umfasst.
7. Verfahren zur Herstellung einer flüssigen pharmazeutischen Zusammensetzung gemäß mindestens einem der Ansprüche 1 bis 6, bei dem die flüssige pharmazeutische Zusammensetzung Ethylendiamintetraessigsäure-Natriumsalz (EDTA) oder ein EDTA-Analogon umfasst, wobei die relative Gewichtsmenge (% w/w) an EDTA optional im Bereich von ungefähr 0,01 bis 0,10 liegt.

Revendications

1. Procédé de fabrication d'une composition pharmaceutique liquide sans un processus de lyophilisation, dans lequel la composition pharmaceutique liquide comprend :
 - (i) une toxine botulique ;
 - (ii) du poloxamère 188 ;

(iii) de la méthionine ; et

(iv) du tréhalose

dans lequel la composition comprend un tampon, dans lequel le tampon est de l'histidine, et dans lequel le pH est dans la plage de 5-7,

dans lequel les quantités en poids relatives (% p/p) de tréhalose, de poloxamère 188, de méthionine sont dans les plages suivantes :

tréhalose ou saccharose	1 à 10
poloxamère 188	0,5 à 5
méthionine	0,1 à 0,3

2. Procédé de fabrication d'une composition pharmaceutique liquide selon la revendication 1, dans lequel la composition pharmaceutique liquide comprend en outre du NaCl, et/ou de l'EDTA, dans laquelle les quantités en poids relatives (% p/p) de NaCl et de l'EDTA sont facultativement dans les plages suivantes :

NaCl	0,1 à 10 et/ou
EDTA	0,01 à 0,1.

3. Procédé de fabrication d'une composition pharmaceutique liquide selon la revendication 1, dans lequel les quantités en poids relatives (% p/p) de tréhalose, de poloxamère 188, de méthionine, et facultativement de NaCl, et facultativement d'EDTA dans la composition pharmaceutique liquide sont :

a)

tréhalose	7 à 9
poloxamère 188	3,5 à 4,5
méthionine	0,15 à 0,25;

et sont de préférence

tréhalose	8
poloxamère 188	4
méthionine	0,2; ou

b)

tréhalose	7 à 9
poloxamère 188	0,5 à 0,7
méthionine	0,15 à 0,25;

et sont de préférence

tréhalose	8
poloxamère 188	0,6
méthionine	0,2; ou

c)

tréhalose	1 à 3
poloxamère 188	3,5 à 4,5

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(suite)

méthionine	0,15 à 0,25
NaCl	0,4 à 0,8 ;

et sont de préférence

tréhalose	2
poloxamère 188	4
méthionine	0,2
NaCl	0,6 ; ou

d)

tréhalose	7 à 9
poloxamère 188	3,5 à 4,5
méthionine	0,15 à 0,25
EDTA	0,01 à 0,05 ;

et sont de préférence

tréhalose	8
poloxamère 188	4
méthionine	0,2
EDTA	0,03

4. Procédé de fabrication d'une composition pharmaceutique liquide selon l'une quelconque des revendications 1 à 3, dans lequel dans la composition pharmaceutique liquide, la concentration d'histidine est de préférence de 20 mM, et dans lequel dans la composition pharmaceutique liquide, le pH est de préférence dans la plage de 5,5 à 6.
5. Procédé de fabrication d'une composition pharmaceutique liquide selon l'une quelconque des revendications 1 à 4, dans lequel la composition pharmaceutique liquide consiste en une ou plusieurs toxines botuliques, du tréhalose, du poloxamère 188, de la méthionine, un tampon, dans lequel le tampon est de l'histidine, et facultativement du NaCl, et facultativement de l'EDTA.
6. Procédé de fabrication d'une composition pharmaceutique liquide selon l'une quelconque des revendications précédentes, dans lequel la composition pharmaceutique liquide ne comprend pas de protéine d'origine animale.
7. Procédé de fabrication d'une composition pharmaceutique liquide selon l'une quelconque des revendications 1 à 6, dans lequel la composition pharmaceutique liquide comprend du sel de sodium d'acide éthylènediaminetétracétique (EDTA) ou un analogue d'EDTA, dans lequel la quantité en poids relative (% p/p) d'EDTA se situe facultativement dans la plage de 0,01 à 0,10.

REFERENCES CITED IN THE DESCRIPTION

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